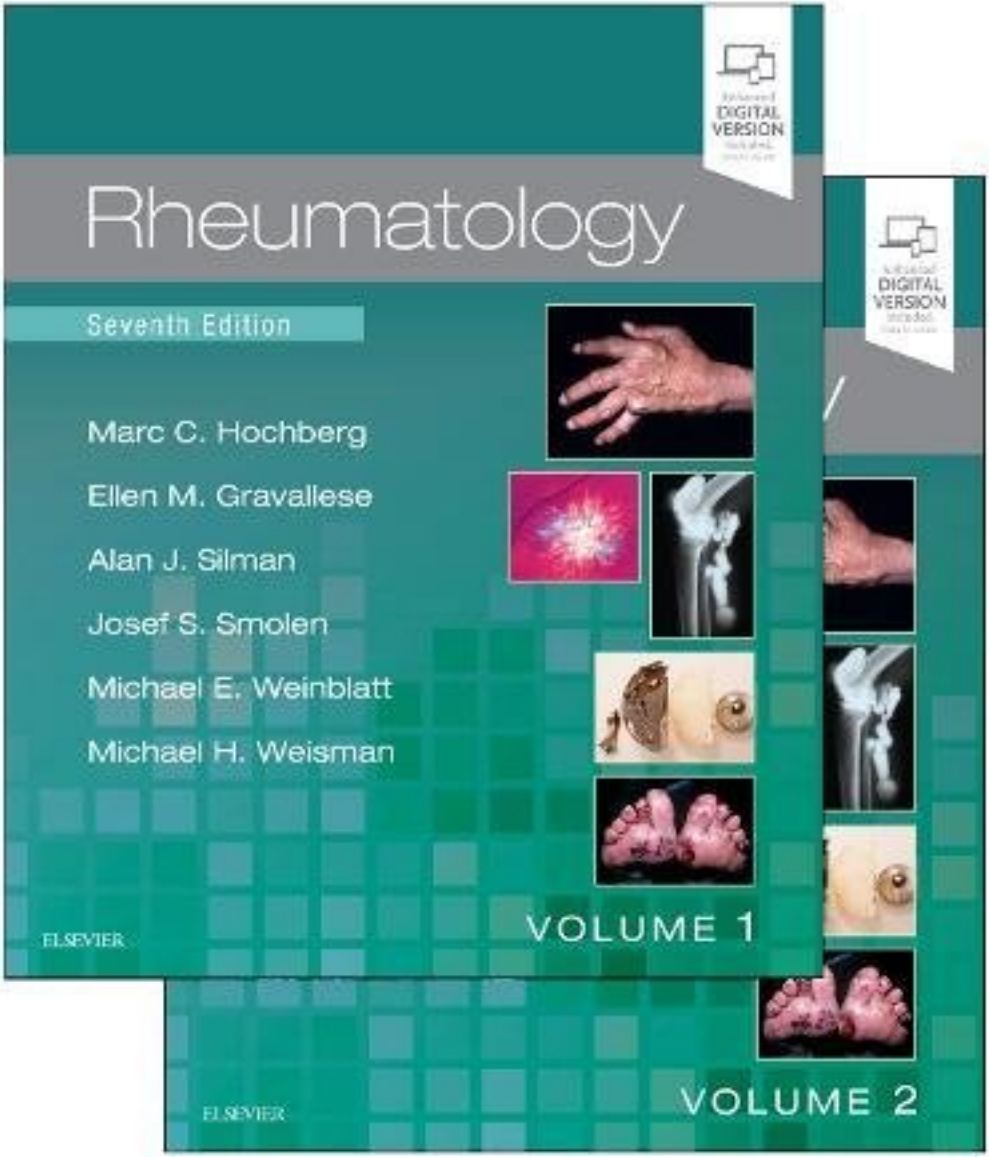
DIGITAL VERSION
Rheumatology
Seventh Edition
Marc C. Hochberg
Ellen M. Gravallese
Alan J. Silman
Josef S. Smolen
Michael E. Weinblatt
Michael H. Weisman
ELSEVIER
VOLUME 1
DIGITAL VERSION
ELSEVIER
VOLUME 2

2-Volume Set

Rheumatology

Rheumatology

Seventh Edition

MARC C. HOCHBERG, MD, MPH, MACP, MACR
Professor of Medicine and Epidemiology and Public Health
Head, Division of Rheumatology and Clinical Immunology
Vice Chair, Department of Medicine
University of Maryland School of Medicine
Director, Gerontology Research Education and Clinical Center
Veterans Affairs Maryland Health Care System
Baltimore, Maryland

ELLEN M. GRAVALLESE, MD
Myles J. McDonough Chair in Rheumatology
Chief, Division of Rheumatology
Department of Medicine
University of Massachusetts Memorial Health Care
Professor of Medicine
University of Massachusetts Medical School
Worcester, Massachusetts

ALAN J. SILMAN, MD, FRCP, FMedSci
Professor of Musculoskeletal Health
Nuffield Department off Orthopaedics, Rheumatology and Musculoskeletal Sciences
Oxford University
United Kingdom

JOSEF S. SMOLEN, MD, FRCP
Professor of Medicine
Chairman, Department of Medicine III
Division of Rheumatology
Medical University of Vienna
Chairman, Second Department of Medicine
Center for Rheumatic Diseases
Hietzing Hospital
Vienna, Austria

MICHAEL E. WEINBLATT, MD, FRCP, MACR
John R. and Eileen K. Riedman Professor of Medicine
Harvard Medical School
R. Bruce and Joan M. Mickey Distinguished Chair in Rheumatology
Division of Rheumatology, Immunology, and Allergy
Brigham and Women's Hospital
Boston, Massachusetts

MICHAEL H. WEISMAN, MD
Cedars-Sinai Chair in Rheumatology
Director, Division of Rheumatology
Professor of Medicine
Cedars-Sinai Medical Center
Distinguished Professor of Medicine
David Geffen School of Medicine at University of California, Los Angeles
Los Angeles, California

ELSEVIER

ELSEVIER

1600 John F. Kennedy Blvd.
Ste 1800
Philadelphia, PA 19103-2899

RHEUMATOLOGY, 7th edition ISBN: 978-0-7020-6865-2

Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

With respect to any drug or pharmaceutical products identified, readers are advised to check the most current information provided (i) on procedures featured or (ii) by the manufacturer of each product to be administered, to verify the recommended dose or formula, the method and duration of administration, and contraindications. It is the responsibility of practitioners, relying on their own experience and knowledge of their patients, to make diagnoses, to determine dosages and the best treatment for each individual patient, and to take all appropriate safety precautions.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Library of Congress Cataloging-in-Publication Data

Names: Hochberg, Marc C., editor.
Title: Rheumatology / [edited by] Marc C. Hochberg, Ellen M. Gravallese, Alan J. Silman, Josef S. Smolen, Michael E. Weinblatt, Michael H. Weisman.
Other titles: Rheumatology (Hochberg)
Description: Seventh edition. | Philadelphia, PA : Elsevier, Inc., [2019] | Includes bibliographical references and index.
Identifiers: LCCN 2017007320 | ISBN 9780702068652 (hardcover : alk. paper)
Subjects: | MESH: Rheumatic Diseases | Antirheumatic Agents–therapeutic use
Classification: LCC RC927 | NLM WE 544 | DDC 616.7/23–dc23 LC record available at https://lccn.loc.gov/2017007320

Content Strategist: Nancy Anastasi Duffy
Content Development Specialist: Jennifer Shreiner
Publishing Services Manager: Patricia Tannian
Project Manager: Ted Rodgers
Design Direction: Bridget Hoette

Printed in China

Last digit is the print number: 9 8 7 6 5 4 3 2 1

To our parents (living or of blessed memory) and our spouses, children, and grandchildren

Susan Hochberg, Francine, Jeffrey, and Eleanor (Nora) Zoe Giuffrida, and Jennifer Hochberg

M. Timothy Hresko, Andrew (Drew) and Gregory Hresko

Ruth Silman, Joanna, Timothy, and Daniel Silman

Alice Smolen, Eva, Daniel, Sami, and Ilona Hruschka, Nina Smolen-Wilson, Etienne, Anna, and James Jandi Wilson, Daniel Smolen and Catherine McKenzie, and Alexander Smolen and Meeri Parikka

Barbara Weinblatt, Hillary, Jason, Sidney Allen and Annika Gray Chapman, and Courtney and Michael Fasciano

Betsy Weisman, Greg, Nicole, Mia, and Joseph Weisman, Lisa, Andrew, David, and Thomas Cope, and Annie, Bill, Caroline, and Dorothy Macomber

Contributors

Abby G. Abelson, MD
Clinical Assistant Professor of Medicine
Chair, Department of Rheumatic and Immunologic Diseases
Cleveland Clinic Lerner College of Medicine
Case Western Reserve University
Cleveland, Ohio
Chapter 199, Management of osteoporosis

Steven B. Abramson, MD
Frederick H. King Professor of Internal Medicine
Chair, Department of Medicine
Professor of Medicine and Pathology
New York University Langone Medical Center
New York, New York
Chapter 24, The microbiome in rheumatic diseases

Jonathan D. Adachi, BSc, MD, FRCPC
Alliance for Better Bone Health Chair in Rheumatology
Professor, Department of Medicine
McMaster University Michael G. DeGroote School of Medicine
St Joseph's Healthcare
Hamilton, Ontario, Canada
Chapter 200, Glucocorticoid-induced osteoporosis

Michael A. Adams, PhD
Professor of Biomechanics
Centre for Applied Anatomy
University of Bristol
Bristol, United Kingdom;
Visiting Professor
Sir Run Run Shaw Hospital
Zheijang University
Hangzhou, China
Chapter 7, Biomechanics of spinal degeneration

Rohit Aggarwal, MD, MS
Associate Professor of Medicine
Division of Rheumatology and Clinical Immunology
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania
Chapter 156, Classification, epidemiology, and clinical features of inflammatory muscle disease

Prof. Dr. Thomas Aigner
Institut für Pathologie
Klinikum Coburg
Coburg, Germany
Chapter 183, Pathogenesis and pathology of osteoarthritis

Shizuo Akira, MD, PhD
Professor
Laboratory of Host Defense
WPI Immunology Frontier Research Center
Osaka University
Osaka, Japan
Chapter 9, Principles of innate immunity

Daniel A. Albert, MD, FACR
Professor of Medicine and Pediatrics
Divisions of Rheumatology
Dartmouth Hitchcock Medical Center
Lebanon, New Hampshire
Chapter 120, Inflammatory back pain

Daniel Aletaha, MD, MSc
Associate Professor of Internal Medicine
Division of Rheumatology
Medical University of Vienna
Vienna, Austria
Chapter 101, Assessment of the patient with rheumatoid arthritis and the measurement of outcomes

Ilias Alevizos, DMD, MMSc, MPH, PhD
Investigator, Sjögren's Syndrome Clinic
Molecular Physiology and Therapeutics Branch
National Institute of Dental and Craniofacial Research
National Institutes of Health
Bethesda, Maryland
Chapter 147, Sjögren syndrome

Kavitta B. Allem, MD
Physician
Division of Rheumatology
Scripps Clinic
La Jolla, California
Chapter 67, Immunosuppressive agents: cyclosporine, cyclophosphamide, azathioprine, mycophenolate mofetil, and tacrolimus

Mohammed Almehthel, MD, ABIM, FRCPC
Clinical Assistant Professor of Medicine
Division of Endocrinology
University of British Columbia
Vancouver, Canada
Chapter 51, Dual x-ray absorptiometry and measurement of bone

Mohamed Almohaya, MD
Endocrinologist
Obesity, Endocrine and Metabolism Center
King Fahad Medical City
Riyadh, Saudi Arabia
Clinical Fellow, Metabolic Bone Disorders
Endocrinology Department
University of British Columbia
Vancouver, Canada
Chapter 51, Dual x-ray absorptiometry and measurement of bone

Elena Riera Alonso, MD
Division of Rheumatology
Hospital Mútua Terrassa
Barcelona, Spain
Chapter 173, Adult-onset Still disease

Mary-Carmen Amigo, MD, FACP
Professor and Head
Rheumatology Service
American British Cowdray Medical Center
Mexico City, Mexico
Chapter 148, Antiphospholipid syndrome: pathogenesis, diagnosis, and management

Nigel K. Arden, MBBS, FRCP, MSc, MD
Professor
Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Sciences
University of Oxford
Oxford, United Kingdom;
Professor in Rheumatic Diseases and Consultant Rheumatologist
Medical Research Council
University of Southampton
Southampton, United Kingdom
Chapter 188, Management of osteoarthritis

Martin Aringer, MD
Professor of Medicine (Rheumatology)
Department of Medicine III, Division of Rheumatology
University Clinical Center Carl Gustav Carus at the TU Dresden
Dresden, Germany
Chapter 11, Signal transduction in immune cells

Elizabeth V. Arkema, ScM, ScD
Assistant Professor of Clinical Epidemiology
Department of Medicine
Karolinska Institutet
Stockholm, Sweden
Chapter 25, Principles of epidemiology

Dana P. Ascherman, MD
Associate Professor of Medicine
Division of Rheumatology
University of Miami Miller School of Medicine
Miami, Florida
Chapter 156, Classification, epidemiology, and clinical features of inflammatory muscle disease

Sergei P. Atamas, MD, PhD
Professor of Medicine and Microbiology & Immunology
University of Maryland School of Medicine;
Research Health Scientist
Baltimore VA Medical Center
Baltimore, Maryland
Chapter 10, Principles of adaptive immunity

Timothy J. Atkinson, PharmD
Clinical Pharmacy Specialist, Pain Management
Pharmacy Service
VA Tennessee Valley Healthcare System
Murfreesboro, Tennessee
Chapter 62, Principles of pharmacologic pain management

Maha A. Azeez, MB BCh BAO, BSc, MD, MRCPI
Nuffield Institute of Orthopedics, Rheumatology, and Musculoskeletal Sciences
University of Oxford
Botnar Research Centre
Headington, United Kingdom
Chapter 72, Tumor necrosis factor inhibitors

Pedro M. Azevedo, MD, PhD
Assistant Professor of Rheumatology
Evangelical University Hospital of Curitiba
Curitiba, Brazil
Chapter 117, Acute rheumatic fever

Alan N. Baer, MD, FACP
Associate Professor of Medicine
Division of Rheumatology
Director, Jerome L. Greene Sjögren's Syndrome Center
Johns Hopkins University;
Clinical Director
Johns Hopkins Rheumatology
Good Samaritan Hospital
Baltimore, Maryland
Chapter 147, Sjögren syndrome

Dominique Baeten, MD, PhD
Professor of Rheumatology
Department of Clinical Immunology and Rheumatology
University of Amsterdam
Academic Medical Center
Amsterdam, The Netherlands
Chapter 122, Pathogenesis and pathophysiology of axial spondyoarthritis

Colin P. Baines, MBChB, MRCP(UK)
Specialty Doctor
Departments of General and Vascular Medicine
Ninewells Hospital and Medical School
Dundee, United Kingdom
Chapter 154, Raynaud phenomenon

Alejandro Balsa, MD, PhD
Head, Department of Rheumatology
Hospital Universitario La Paz
Associate Professor of Rheumatology
Department of Medicine
Universidad Autonoma
Madrid, Spain
Chapter 113, Bacterial arthritis

Xenofon Baraliakos, MD
Rheumazentrum Ruhrgebiet
Ruhr-University Bochum
Herne, Germany
Chapter 125, Imaging of spondyloarthritis

Thomas Bardin, MD
Professor of Rheumatology
Université Paris 7;
Head, Department of Rheumatology
Hôpital Lariboisière
Paris, France
Chapter 214, Miscellaneous arthropathies

Jammie K. Barnes, MD
Assistant Professor of Medicine
Division of Rheumatology
University of Texas Health Science Center Houston
McGovern School of Medicine
Houston, Texas
Chapter 149, Classification and epidemiology of systemic sclerosis (scleroderma)

Les Barnsley, B.Med(Hons), Grad.Dip.Epi., PhD, FRACP, FAFRM(RACP)
Associate Professor of Medicine
Sydney University
Sydney, Australia;
Head, Department of Rheumatology
Concord Hospital
Concord, Australia
Chapter 80, Neck pain

Andrew J. Barr, MBBS, PhD
Clinical Research Fellow
Leeds Institute of Rheumatic and Musculoskeletal Medicine
University of Leeds
Chapel Allerton Hospital
Leeds, United Kingdom
Chapter 185, Imaging of osteoarthritis

Joan M. Bathon, MD
Professor of Medicine
Division of Rheumatology
Columbia University College of Physicians and Surgeons
New York, New York
Chapter 103, Management of established rheumatoid arthritis

Angela Bauch, PhD
CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences
Vienna, Austria
Chapter 22, Proteomics

Anne-Christine Bay-Jensen, mMBS, MSc, PhD
Head, Department of Rheumatology
Division of Biomarkers and Research
Nordic Bioscience
Herlev, Denmark
Chapter 198, Biochemical markers in bone disease

Jill J.F. Belch, MBChB, MD (Hons), FRCP, FRS
Professor of Vascular Medicine
Division of Clinical and Molecular Medicine
Ninewells Hospital and Medical School
Dundee, United Kingdom
Chapter 154, Raynaud phenomenon

Nicholas Bellamy, MD, MSc, MBA, DSc, FRCP(C), FRCP (Glasg, Edin), FACP, FRACP
Emeritus Professor
Centre for Chronic Disease
The University of Queensland School of Medicine
Herston, Australia
Chapter 26, Principles of clinical outcome assessment

Teresita Bellido, PhD
Professor
Departments of Anatomy and Cell Biology and Internal Medicine (Endocrinology)
Indiana University School of Medicine
Roudebush VA Medical Center
Indianapolis, Indiana
Chapter 3, Bone structure and function

Michael Benjamin, PhD†
Professor Emeritus
School of Biosciences
Cardiff University
Cardiff, United Kingdom
Chapter 132, Enthesopathies

Kim L. Bennell, BAppSci(physio), PhD
Professor of Physiotherapy
Director, Centre for Health, Exercise, and Sports Medicine
University of Melbourne
Melbourne, Australia
Chapter 55, Principles of rehabilitation: physical and occupational therapy

Michael W. Beresford, MBChB, PhD
Brough Chair, Professor of Child Health,
Institute of Translational Medicine
University of Liverpool
Honorary Consultant Paediatric Rheumatologist
Alder Hey Children's NHS Foundation Trust
Liverpool, United Kingdom
Chapter 111, Connective tissue diseases in children

Brian Berman, MD
Professor of Family and Community Medicine
Director, Center for Integrative Medicine
University of Maryland School of Medicine
Baltimore, Maryland
Chapter 58, Complementary and alternative medicine

Bonnie L. Bermas, MD
Assistant Professor of Medicine
Division of Rheumatology
Harvard Medical School
Director, Lupus Center
Brigham and Women's Hospital
Boston, Massachusetts
Chapter 59, Drugs and pregnancy

George Bertsias, MD, PhD
Assistant Professor in Rheumatology-Clinical Immunology
University of Crete;
Rheumatology, Clinical Immunology and Allergy
University Hospital of Iraklio
Iraklio, Greece
Chapter 145, Management of renal lupus

Sonal Bhalla, MD
Fellow, Department of Rheumatology
Dartmouth Hitchcock Medical Center
Lebanon, New Hampshire
Chapter 120, Inflammatory back pain

Yelda Bilginer, MD
Associate Professor of Pediatric Nephrology and Rheumatology
Hacettepe University Faculty of Medicine
Ankara, Turkey
Chapter 169, IgA vasculitis (Henoch-Schoenlein Purpura)

Julius Birnbaum, MD
Assistant Professor of Medicine
Assistant Professor of Neurology
Associate Director, Jerome L. Greene Sjögren's Syndrome Center
Johns Hopkins University School of Medicine
Baltimore, Maryland
Chapter 41, The nervous system in rheumatic disease

Jane F. Bleasel, MB BS, PhD, MHPE, FRACP
Professor of Rheumatology
Royal Prince Alfred Hospital
Sydney Medical School
University of Sydney
Sydney, Australia
Chapter 208, Hemophilia and von Willebrand disease

†Deceased.

Joel A. Block, MD
The Willard L. Wood MD Professor and Director
Division of Rheumatology
Rush University Medical Center
Chicago, Illinois
Chapter 181, Clinical features of osteoarthritis

Ashley W. Blom, MBChB, MD, PhD, FRCS
Professor of Orthopaedic Surgery
Musculoskeletal Research Unit
University of Bristol
Bristol, United Kingdom
Chapter 61, Long-term complications of total hip and knee replacement

Markus Böhm, MD
Professor of Dermatology
University of Münster
Münster, Germany
Chapter 35, The skin in rheumatic disease

Michael Bonelli, PhD
Fellow
Molecular Immunology and Inflammation Branch
National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institutes of Health
Bethesda, Maryland
Chapter 11, Signal transduction in immune cells

Grace Borchert
Research Assistant
Translational Research Institute
The University of Queensland Diamantina Institute
Brisbane, Australia
Chapter 124, Animal models of spondyloarthritis

Christina A. Boros, MBBS, PhD, FRACP
Honorary Research Fellow
Division of Infection, Inflammation and Rheumatology
UCL Great Ormond Street Institute of Child Health
London, United Kingdom;
Senior Lecturer
Discipline of Paediatrics and Reproductive Health
University of Adelaide
Head, Rheumatology Unit
Women's and Children's Hospital
Adelaide, Australia
Chapter 111, Connective tissue diseases in children

Dimitrios T. Boumpas, MD, FACP
Professor of Internal Medicine
National and Kapodistrian University of Athens Medical School
Sotiria General Hospital for Pulmonary and Heart Diseases;
Rheumatology and Clinical Immunology
4th Department of Internal Medicine
Attikon University Hospital;
Affiliated Investigator
Immunobiology
Biomedical Research Foundation of the Academy of Athens
Athens, Greece
Chapter 145, Management of renal lupus

Aline Bozec, PhD
Professor of Rheumatology and Immunology
Department of Internal Medicine 3
University of Erlangen-Nuremberg Faculty of Medicine
Erlangen, Germany
Chapter 16, Osteoimmunology

Richard D. Brasington, Jr., MD
Professor of Medicine
Division of Rheumatology
Washington University School of Medicine in St. Louis
St. Louis, Missouri
Chapter 94, Clinical features of rheumatoid arthritis

Prof. Dr. Jürgen Braun
Rheumazentrum Ruhrgebiet
Ruhr-University Bochum
Herne, Germany
Chapter 125, Imaging of spondyloarthritis

Elisabeth Brouwer, MD, PhD
Department of Rheumatology and Clinical Immunology
University of Groningen, University Medical Center Groningen
Groningen, The Netherlands
Chapter 162, Biology and immunopathogenesis of vasculitis

Jacques P. Brown, MD, FRCPC
Clinical Professor of Medicine
Université Laval;
Division of Infectious Disease and Immunity
CHU de Québec Research Centre
Québec City, Canada
Chapter 203, Paget disease of bone

Matthew A. Brown, MBBS, MD, FRACP, FAHMS, FAA
Professor of Immunogenetics
Director of Genomics
Queensland University of Technology
Institute of Health and Biomedical Innovation
Translational Research Institute
Princess Alexandra Hospital
Brisbane, Australia
Chapter 123, Genetics of axial spondyloarthritis

Ian N. Bruce, MD, FRCP
Professor of Rheumatology
Arthritis Research UK Epidemiology Unit
The University of Manchester
Manchester, United Kingdom
Chapter 128, Clinical features of psoriatic arthritis

Maya H. Buch, MBChB, FRCP, PhD
Associate Professor and Honorary Consultant Rheumatologist
Section Head, Clinical Musculoskeletal Medicine
Deputy Director, Leeds Institute of Rheumatic and Musculoskeletal Medicine
University of Leeds
Leeds, United Kingdom
Chapter 102, Management of early rheumatoid arthritis

Christopher D. Buckley, MBBS, DPhil
Professor of Medicine
Rheumatology Research Group
Institute of Inflammation and Ageing
University of Birmingham
Birmingham, United Kingdom
Chapter 1, The synovium

William D. Bugbee, MD
Attending Physician
Division of Orthopaedic Surgery
Scripps Clinic
La Jolla, California;
Professor of Orthopaedic Surgery
University of California, San Diego
San Diego, California
Chapter 85, The hip

Gerd-Rüdiger Burmester, MD
Professor of Medicine
Division of Rheumatology and Clinical Immunology
Charité-Universitätsmedizin Berlin
Berlin, Germany
Chapter 33, Laboratory tests in rheumatic disorders

Jane C. Burns, MD
Professor of Pediatrics
Director, Kawasaki Disease Research Center
University of California San Diego School of Medicine
La Jolla, California;
Director, Kawasaki Disease Clinic
Rady Children's Hospital-San Diego
San Diego, California
Chapter 168, Kawasaki disease

David B. Burr, PhD
Distinguished Professor
Associate Vice Chancellor for Research (IUPUI)
Department of Anatomy and Cell Biology, Indiana University School of Medicine
Department of Biomedical Engineering, IUPUI
Indianapolis, Indiana
Chapter 3, Bone structure and function

Frank Buttgereit, MD
Professor of Rheumatology and Clinical Immunology
Charité University Medicine Berlin
Berlin, Germany
Chapter 64, Systemic glucocorticoids in rheumatology

Vivian P. Bykerk, MD
Associate Professor of Medicine
Hospital for Special Surgery
New York, New York
Chapter 65, Synthetic disease-modifying antirheumatic drugs and leflunomide

Leonard H. Calabrese, DO
Professor of Medicine
Cleveland Clinic Lerner College of Medicine
Case Western University
Vice Chairman, Department of Rheumatic and Immunological Diseases
R.J. Fasenmyer Chair of Clinical Immunology
Theodore F. Classen DO Chair of Osteopathic Research and Education
Cleveland Clinic Foundation
Cleveland, Ohio
Chapter 115, Viral infections

Jeffrey P. Callen, MD
Professor of Medicine (Dermatology)
Chief, Division of Dermatology
University of Louisville School of Medicine
Louisville, Kentucky
Chapter 170, Cutaneous vasculitis and panniculitis

Andrew J. Carr, DSc, MA, ChM, FRCS
Nuffield Professor of Orthopaedic Surgery
Head, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences
Director, Botnar Research Centre
University of Oxford
Oxford, United Kingdom
Chapter 4, Tendons and ligaments

Graeme J. Carroll, MBBS, MD, FRACP
Assistant Professor of Medicine
University of Notre Dame School of Medicine
Fremantle, Australia;
Consultant Physician
Division of Rheumatology
Fiona Stanley Hospital
Perth, Australia
Chapter 210, Hemochromatosis

Sabrina Cavallo, BSc (OT), MSc
Ecole de Santé Publique
Université de Montréal;
Occupational Therapist
Department of Occupational Therapy
Montreal Children's Hospital of the McGill University Health Centre
Montréal, Canada
Chapter 112, Rehabilitation and psychosocial issues in juvenile idiopathic arthritis

Ricard Cervera, MD, PhD, FRCP
Head, Department of Autoimmune Diseases
Hospital Clínic
University of Barcelona
Barcelona, Catalonia, Spain
Chapter 143, Management of nonrenal and non–central nervous system lupus

Prateek Chaudhary, DO, MPH
Fellow
Division of Rheumatology and Immunology
Duke University Medical Center
Durham, North Carolina
Chapter 146, Systemic lupus erythematosus in pregnant patients and neonatal lupus

Hyon K. Choi, MD, DrPH
Professor of Medicine
Division of Rheumatology, Allergy, and Immunology
Harvard Medical School;
Director, Gout and Crystal Arthropathy Center
Director, Clinical Epidemiology and Health Outcomes
Massachusetts General Hospital
Boston, Massachusetts
Chapter 189, Epidemiology and classification of gout

Ernest H. Choy, MD, FRCP
Professor of Medicine
Cardiff University School of Medicine
Section of Rheumatology
Institute of Infection and Immunity
Cardiff, United Kingdom
Chapter 74, Inhibitors of T-cell co-stimulation

Lisa Christopher-Stine, MD, MPH
Associate Professor of Medicine (Rheumatology) and Neurology
Director, Johns Hopkins Myositis Center
Johns Hopkins University School of Medicine
Baltimore, Maryland
Chapter 160, Metabolic, drug-induced, and other noninflammatory myopathies

Alvina D. Chu, MD
Adjunct Clinical Instructor
Division of Immunology and Rheumatology
Stanford University School of Medicine
Palo Alto, California
Pharmacyclics LLC, an AbbVie Company
Sunnyvale, California
Chapter 142, Assessing disease activity and outcomes in systemic lupus erythematosus

Lorinda Chung, MD, MS
Associate Professor of Medicine and Dermatology
Division of Immunology and Rheumatology
Stanford University School of Medicine
Stanford, California
Chapter 158, Clinical significance of autoantibodies in inflammatory muscle disease

Francesco Ciccia, MD, PhD
Dipartimento Biomedico di Medicina Interna e Specialistica
Università degli Studi di Palermo
Palermo, Italy
Chapter 166, Polymyalgia rheumatica and giant cell arteritis

Daniel J. Clauw, MD
Professor of Anesthesiology, Medicine (Rheumatology) and Psychiatry
Director, Chronic Pain and Fatigue Research Center
University of Michigan Medical School
Ann Arbor, Michigan
Chapter 91, Fibromyalgia and related syndromes

Jacqui Clinch, MBBS, MRCP, FRCPCH
Consultant in Paediatric Rheumatology
Bristol Royal Hospital for Children
Bristol, United Kingdom;
Consultant in Paediatric Pain
Bath Centre for Pain Services
Royal National Hospital for Rheumatic Diseases
Bath, United Kingdom
Chapter 216, Hypermobility syndrome

Megan E.B. Clowse, MD, MPH
Assistant Professor of Medicine
Division of Rheumatology and Immunology
Duke University Medical Center
Durham, North Carolina
Chapter 146, Systemic lupus erythematosus in pregnant patients and neonatal lupus

J. Gerard Coghlan, MD, FRCP
Consultant Cardiologist
Royal Free Hospital
London, United Kingdom
Chapter 37, The heart in rheumatic disease

Robert A. Colbert, MD, PhD
Senior Investigator
National Institute of Arthritis, Musculoskeletal and Skin Diseases
National Institutes of Health
Bethesda, Maryland
Chapter 108, Etiology and pathogenesis of juvenile idiopathic arthritis

Philip G. Conaghan, MBBS, PhD, FRACP, FRCP
Professor of Musculoskeletal Medicine
Leeds Institute of Rheumatic and Musculoskeletal Medicine
University of Leeds;
Deputy Director
National Institute for Health Research
Leeds Musculoskeletal Biomedical Research Unit
Leeds, United Kingdom
Chapter 185, Imaging of osteoarthritis

Cyrus Cooper, FMedSci
Professor and Director
MRC Lifecourse Epidemiology Unit
University of Southampton
Southampton, United Kingdom
Chapter 195, Epidemiology and classification of osteoporosis

Robert G. Cooper, MD, FRCP
Professor of Medicine (Muscle and Rheumatology)
Institute of Ageing and Chronic Disease
University of Liverpool
Liverpool, United Kingdom
Chapter 42, The muscles in rheumatic disease

Karen H. Costenbader, MD, MPH
Co-Director, Lupus Center
Division of Rheumatology, Immunology and Allergy
Brigham and Women's Hospital;
Associate Professor of Medicine
Harvard Medical School
Boston, Massachusetts
Chapter 133, Epidemiology and classification of systemic lupus erythematosus

George L.D. Cox, BMedSci, BMBS, MD, FRCS(T&O)
Consultant
Departments of Trauma and Orthopaedics
University Hospital Southampton
Southampton, United Kingdom
Chapter 82, The shoulder

Paul Creamer, MD, FRCP
Consultant and Senior Clinical Lecturer
Department of Rheumatology
North Bristol Trust
Bristol, United Kingdom
Chapter 205, Neuropathic arthropathy

Bruce N. Cronstein, MD
Paul R. Esserman Professor of Medicine
Division of Rheumatology
New York University School of Medicine
New York, New York
Chapter 79, Precision medicine and pharmacogenomics in rheumatology

Raymond Cross, MD, MS
Associate Professor of Medicine
University of Maryland School of Medicine
Baltimore, Maryland
Chapter 39, The gastrointestinal tract and rheumatic disease

Maurizio Cutolo, MD
Professor of Rheumatology and Internal Medicine
Director, Research Laboratories and Academic Unit of Clinical Rheumatology
Director, Postgraduate Academic School of Rheumatology
University of Genova
Genova, Italy
Chapter 15, Neuroendocrine immune crosstalk in chronic inflammatory systemic diseases

Chris D'Adamo, PhD
Assistant Professor
Department of Family and Community Medicine
Assistant Professor
Department of Epidemiology and Public Health
Director of Research
Center for Integrative Medicine
University of Maryland School of Medicine
Baltimore, Maryland
Chapter 58, Complementary and alternative medicine

Vivette D. D'Agati, MD
Professor of Pathology
Columbia University College of Physicians and Surgeons
Director, Renal Pathology Laboratory
Columbia University Medical Center
New York, New York
Chapter 137, Immunopathology of systemic lupus erythematosus

David I. Daikh, MD, PhD
Professor of Medicine
University of California, San Francisco;
Chief, Arthritis Section
San Francisco VA Medical Center
San Francisco, California
Chapter 138, Animal models of systemic lupus erythematosus

Stephanie G. Dakin, PhD, BVetMed, MRCVS
Oxford UCB Prize Fellow
Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences
University of Oxford
Oxford, United Kingdom
Chapter 4, Tendons and ligaments

Nicola Dalbeth, MBChB, MD, FRACP
Professor and Rheumatologist
Department of Medicine
University of Auckland
Auckland, New Zealand
Chapter 190, Etiology and pathogenesis of gout

Aileen M. Davis, BScPT, MSc, PhD
Senior Scientist, Health Care, and Outcomes Research
Toronto Western Research Institute
University Health Network;
Professor
Institute of Health Policy, Management, and Evaluation
Department of Physical Therapy and Graduate Department of Rehabilitation Science
University of Toronto
Toronto, Ontario, Canada
Chapter 186, Assessment of the patient with osteoarthritis and measurement of outcomes

David P. D'Cruz, MD, FRCP
Consultant Rheumatologist
The Louise Coote Lupus Unit
St. Thomas' Hospital
London, United Kingdom
Chapter 37, The heart in rheumatic disease

Karel De Ceulaer, MD, Dipl Rheum
Consultant Rheumatologist
Department of Medicine
University of the West Indies
Kingston, Jamaica
Chapter 209, Sickle cell disease and other hemoglobinopathies

Adriana A. de Jesus, MD, PhD
Staff Scientist
Translational Autoinflammatory Diseases Studies
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland
Chapter 174, Monogenic autoinflammatory diseases

Salvatore De Vita, MD
Professor of Rheumatology
Department of Medical Area
University of Udine;
Chief, Rheumatology Clinic
Academic Hospital 'S. Maria della Misericordia'
Udine, Italy
Chapter 171, Cryoglobulinemia

Maarten de Wit, PhD
Department of Medical Humanities
VU University Medical Centre
Amsterdam, The Netherlands
Chapter 52, The patient perspective

Chad L. Deal, MD
Associate Professor of Medicine
Cleveland Clinic Lerner College of Medicine
Case Western Reserve University
Cleveland, Ohio
Chapter 199, Management of osteoporosis

Kevin D. Deane, MD, PhD
Associate Professor of Medicine
Division of Rheumatology
University of Colorado School of Medicine
Aurora, Colorado
Chapter 93, Preclinical rheumatoid arthritis

Paul F. Dellaripa, MD
Associate Professor of Medicine
Harvard Medical School;
Division of Rheumatology
Brigham and Women's Hospital
Boston, Massachusetts
Chapter 38, The lung in rheumatic disease

Elaine Dennison, MB, BChir, MSc, FRCP, PhD
Professor of Musculoskeletal Epidemiology
MRC Lifecourse Epidemiology Unit
Southampton University
Southampton, United Kingdom;
Professor of Clinical Research
School of Biological Sciences
Victoria University
Wellington, New Zealand
Chapter 195, Epidemiology and classification of osteoporosis

Christopher P. Denton, PhD
Professor of Experimental Rheumatology
Consultant Rheumatologist
Centre for Rheumatology
University College London
Royal Free Hospital
London, United Kingdom
Chapter 150, Clinical and serologic features of systemic sclerosis

Paul Dieppe, BSc, MD, FRCP, FFPH
Emeritus Professor of Health and Wellbeing
University of Exeter Medical School
Exeter, United Kingdom
Chapter 57, Placebos, caring, and healing in rheumatology

Patricia Dolan, PhD
Reader in Biomechanics
Centre for Applied Anatomy
University of Bristol
Bristol, United Kingdom;
Visiting Professor
Sir Run Run Shaw Hospital
Zheijang University
Hanghzhou, China
Chapter 7, Biomechanics of spinal degeneration

Tracy J. Doyle, MD, MPH
Instructor in Medicine
Harvard Medical School
Pulmonary and Critical Care Division
Brigham and Women's Hospital
Boston, Massachusetts
Chapter 38, The lung in rheumatic disease

George S.M. Dyer, MD
Assistant Professor of Orthopedic Surgery
Harvard Medical School
Orthopaedic Hand Surgeon
Brigham and Women's Hospital
Boston, Massachusetts
Chapter 84, The wrist and hand

Paul Emery, MD, MA, FMedSci, FRCP
Arthritis Research UK Professor of Rheumatology
Director, Leeds Institute of Rheumatic and Musculoskeletal Medicine
University of Leeds;
Director, NIHR Leeds Musculoskeletal Biomedical Research Centre
Leeds Teaching Hospital NHS Trust
Leeds, United Kingdom
Chapter 75, Inhibitors of B cells

Gerard Espinosa, MD, PhD
Department of Autoimmune Diseases
Hospital Clínic
University of Barcelona
Barcelona, Catalonia, Spain
Chapter 143, Management of nonrenal and non–central nervous system lupus

Luis R. Espinoza, MD
Professor and Chief
Department of Internal Medicine
LSU Health Science Center
Louisiana State University
New Orleans, Louisiana
Chapter 114, Mycobacterial, brucellar, fungal, and parasitic arthritis

Stephen Eyre, PhD
Research Associate
Arthritis Research UK Epidemiology Unit
The University of Manchester
Manchester, United Kingdom
Chapter 28, Principles of genetic epidemiology

Antonis C. Fanouriakis, MD
Research Associate
Rheumatology and Clinical Immunology
4th Dept of Internal Medicine
Attikon University Hospital
Athens, Greece
Chapter 145, Management of renal lupus

Joshua Farber, MD
Senior Investigator
Laboratory of Molecular Immunology, DIR
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland
Chapter 12, Cytokines

Anders Fasth, MD, PhD
Professor, Department of Pediatrics
University of Gothenburg
Consultant, Department of Pediatric Immunology
The Queen Silvia Children's Hospital
Gothenburg, Sweden
Chapter 107, Clinical features of juvenile idiopathic arthritis

Eugen Feist, MD
Professor of Medicine
Division of Rheumatology and Clinical Immunology
Charité-Universitätsmedizin Berlin
Berlin, Germany
Chapter 33, Laboratory tests in rheumatic disorders

Candace H. Feldman, MD, MPH, ScD
Associate Physician
Division of Rheumatology, Immunology and Allergy
Brigham and Women's Hospital;
Assistant Professor of Medicine
Harvard Medical School
Boston, Massachusetts
Chapter 133, Epidemiology and classification of systemic lupus erythematosus

Debbie Feldman, PhD
Professor
Faculty of Medicine, School of Rehabilitation
Université de Montréal
Montréal, Canada
Chapter 112, Rehabilitation and psychosocial issues in juvenile idiopathic arthritis

Andrew Filer, MBChB, PhD
Senior Lecturer
School of Immunity and Infection
The University of Birmingham
Honorary Consultant Rheumatologist
Department of Rheumatology
University Hospitals Birmingham NHS Foundation Trust
Birmingham, United Kingdom
Chapter 1, The synovium

David F. Fiorentino, MD, PhD
Professor
Department of Dermatology
Stanford University School of Medicine
Stanford, California
Chapter 158, Clinical significance of autoantibodies in inflammatory muscle disease

Benjamin A. Fisher, MD(res) MBBS
Senior Clinical Lecturer
Institute of Inflammation and Ageing
University of Birmingham
Honorary Consultant Rheumatologist
University Hospitals Birmingham NHS Trust
Birmingham, United Kingdom
Chapter 69, Biologic agents: monoclonal antibodies and receptor antagonists

John D. Fisk, PhD
Psychologist
Nova Scotia Health Authority
Associate Professor of Psychiatry
Associate Professor Psychology & Neuroscience
Assistant Professor of Medicine
Dalhousie University
Halifax, Nova Scotia, Canada
Chapter 144, Management of central nervous system lupus

Raymond H. Flores, MD
Associate Professor of Medicine
Division of Rheumatology and Clinical Immunology
University of Maryland School of Medicine
Baltimore, Maryland
Chapter 89, Entrapment neuropathies and compartment syndromes

Lindsy Forbess, MD, MSc
Assistant Professor of Medicine
Division of Rheumatology
Cedars-Sinai Medical Center
Los Angeles, California
Chapter 163, Polyarteritis nodosa and Cogan syndrome

David A. Fox, MD
Professor of Medicine
Division of Rheumatology
University of Michigan Medical School
Ann Arbor, Michigan
Chapter 73, Interleukin-17, interleukin-12, and interleukin-23 inhibitors

Anthony J. Freemont, BSc, MB BS, MD, FRCP, FRCPath
Procter Professor of Pathology
Director of MMPathIC
Faculty of Biology, Medicine and Health
University of Manchester
Manchester, United Kingdom
Chapter 34, Synovial fluid analysis

Kevin B. Fricka, MD
Clinical Instructor
Adult Reconstructive Hip and Knee Fellowship
Anderson Orthopaedic Clinic
Alexandria, Virginia
Chapter 85, The hip

Cem Gabay, MD
Professor of Medicine
Head, Division of Rheumatology
University Hospitals of Geneva
Geneva, Switzerland
Chapter 70, Interleukin-1 inhibitors

Massimo Gadina, PhD
Chief
Translational Immunology Section
National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health
National Institutes of Health
Bethesda, Maryland
Chapter 12, Cytokines

Saviana Gandolfo, MD
Rheumatology Clinic
Department of Medical Area
Academic Hospital 'S. Maria della Misericordia'
Udine, Italy
Chapter 171, Cryoglobulinemia

Jeremy Gauntlett-Gilbert, PhD, D.Clin.Psy.
Principal Clinical Psychologist
Bath Centre for Pain Services
Royal United Hospitals
Bath, United Kingdom;
Visiting Fellow
Faculty of Health and Applied Sciences
University of the West of England
Bristol, United Kingdom
Chapter 56, Multi-disciplinary approaches to managing chronic pain in arthritis

Steffen Gay, MD
Professor of Experimental Rheumatology
Lead Physician
Center for Experimental Rheumatology
University Hospital Zürich
Zürich, Switzerland
Chapter 23, Epigenetics

Lianne S. Gensler, MD
Assistant Clinical Professor of Medicine
University of California San Francisco
San Francisco, California
Chapter 121, Clinical features of axial spondyloarthritis

Danielle M. Gerlag, MD, PhD
Associate Professor
Department of Clinical Immunology and Rheumatology
Academic Medical Center, University of Amsterdam
Amsterdam, The Netherlands;
Head, Clinical Unit Cambridge
Department of Research and Development
GlaxoSmithKline
Cambridge, United Kingdom
Chapter 45, Minimally invasive procedures

Garry E. Gold, MD
Professor of Radiology
Stanford School of Medicine
Palo Alto, California
Chapter 47, Functional magnetic resonance imaging

Raphaela Goldbach-Mansky, MD, MHS
Chief, Translational Auto-inflammatory Disease Studies
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland
Chapter 174, Monogenic autoinflammatory diseases

Sarah J. Goldingay, MA, PhD
Lecturer
College of Humanities
University of Exeter
Exeter, United Kingdom
Chapter 57, Placebos, caring, and healing in rheumatology

José A. Gómez-Puerta, MD, PhD, MPH
Grupo de Inmunología Celular e Inmunogenética
Universidad de Antioquia
Medellín, Colombia
Chapter 143, Management of nonrenal and non–central nervous system lupus

Susan M. Goodman, MD
Professor of Clinical Medicine
Weill Cornell Medical School
Attending Physician
Division of Rheumatology
Hospital for Special Surgery
New York, New York
Chapter 60, Perioperative care of the rheumatic disease patient

Caroline Gordon, MD, MA, FRCP
Professor of Rheumatology
Consultant Rheumatologist
Institute of Inflammation and Ageing
University of Birmingham
Birmingham, United Kingdom
Chapter 69, Biologic agents: monoclonal antibodies and receptor antagonists

Sharon M. Gordon, DDS, MPH, PhD
Professor and Chair
Department of Foundational Sciences
Associate Dean for Research
East Carolina University School of Dental Medicine
Greenville, North Carolina
Chapter 88, The temporomandibular joint

Rachel Gorodkin, MBChB, PhD, FRCP
Consultant Rheumatologist
The Kellgren Centre for Rheumatology
Manchester Royal Infirmary;
Honorary Lecturer
Faculty of Medical and Human Sciences
University of Manchester
Manchester, United Kingdom
Chapter 90, Complex regional pain syndrome (reflex sympathetic dystrophy)

Simon Görtz, MD
Chief Resident
Department of Orthopaedic Surgery
University of California, San Diego
San Diego, California
Chapter 85, The hip

Andrew J. Grainger, BM BS, MRCP, FRCR
Consultant and Honorary Senior Lecturer
Department of Musculoskeletal Radiology
Leeds Teaching Hospitals
Leeds, United Kingdom
Chapter 185, Imaging of osteoarthritis

Ellen M. Gravallese, MD
Myles J. McDonough Chair in Rheumatology
Chief, Division of Rheumatology
Department of Medicine
University of Massachusetts Memorial Health Care
Professor of Medicine
University of Massachusetts Medical School
Worcester, Massachusetts
Chapter 100, Pathogenesis and pathology of rheumatoid arthritis

Jeffrey D. Greenberg, MD, MPH
Assistant Professor of Medicine
Division of Rheumatology
New York University School of Medicine
New York, New York
Chapter 79, Precision medicine and pharmacogenomics in rheumatology

Sharon Grieve, MRes Clinical Research, BSc(Hons)
Pain Lead Research Nurse
Complex Regional Pain Syndrome Service
Royal United Hospitals
Bath, United Kingdom;
Visiting Research Fellow
Faculty of Health and Applied Sciences
University of the West of England
Bristol, United Kingdom
Chapter 56, Multi-disciplinary approaches to managing chronic pain in arthritis

Luiza Guilherme, PhD
Professor of Immunology
Heart Institute - InCor
University of São Paulo School of Medicine
Institute for Immunology Investigation
National Institute for Science and Technology
São Paulo, Brazil
Chapter 117, Acute rheumatic fever

Ahmet Gül, MD
Professor of Internal Medicine
Division of Rheumatology
Istanbul University
Istanbul Faculty of Medicine
Istanbul, Turkey
Chapter 167, Behçet disease

Sarthak Gupta, MD
Henry Metzger Scholar
Systemic Autoimmunity Branch
National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institutes of Health
Bethesda, Maryland
Chapter 140, Pathogenesis of systemic lupus erythematosus

Matilda Hagan, MD
Division of Gastroenterology
University of Maryland School of Medicine
Baltimore, Maryland
Chapter 39, The gastrointestinal tract and rheumatic disease

Karlene Hagley, MBBS, MD
Consultant in Internal Medicine
Spanish Town Hospital
St. Catherine, Jamaica;
Associate Lecturer
Department of Medicine
University of the West Indies
Kingston, Jamaica
Chapter 209, Sickle cell disease and other hemoglobinopathies

Shuhong Han, PhD
Assistant Scientist
Division of Rheumatology and Clinical Immunology
University of Florida College of Medicine
Gainesville, Florida
Chapter 139, Autoantibodies in systemic lupus erythematosus

John G. Hanly, MD, FRCP(C)
Professor of Medicine and Pathology
Dalhousie University;
Attending Staff Rheumatologist
Queen Elizabeth II Health Sciences Center
Halifax, Nova Scotia, Canada
Chapter 144, Management of central nervous system lupus

Eric P. Hanson, MD
Senior Clinical Fellow
Immunodeficiency and Inflammation Unit, Autoimmunity Branch
National Institute of Arthritis Musculoskeletal and Skin Disease
National Institutes of Health
Bethesda, Maryland
Chapter 11, Signal transduction in immune cells

Boulos Haraoui, MD, FRCPC
Associate Professor of Medicine
Université de Montréal
Montreal, Québec, Canada
Chapter 65, Synthetic disease-modifying antirheumatic drugs and leflunomide

Sarah A. Hardcastle, MBChB, BSc (Hons), PhD
Specialist Registrar in Rheumatology
Royal National Hospital for Rheumatic Diseases
Bath, United Kingdom
Chapter 196, Clinical features of osteoporosis

John B. Harley, MD, PhD
Professor of Pediatrics
University of Cincinnati College of Medicine;
Director, Center for Autoimmune Genomics and Etiology
Cincinnati Children's Hospital Medical Center
Cincinnati, Ohio
Chapter 136, Genetics of systemic lupus erythematosus

Tayseer G. Haroun, MBBS
Fellow
Division of Rheumatology and Immunology
Duke University Medical Center
Durham, North Carolina
Chapter 146, Systemic lupus erythematosus in pregnant patients and neonatal lupus

Philip J. Hashkes, MD, MSc
Head, Pediatric Rheumatology Unit
Department of Pediatrics
Shaare Zedek Medical Center
Clinical Associate Professor of Pediatrics
Hebrew University Hadassah School of Medicine
Jerusalem, Israel;
Associate Professor of Medicine and Pediatrics
Cleveland Clinic Lerner College of Medicine
Case Western Reserve University
Cleveland, Ohio
Chapter 109, Management of juvenile idiopathic arthritis

Andrew Bassim Hassan, DPhil, FRCP
Head, Sarcoma and TYA Oncology Unit
NHS Department of Oncology
Oxford Haematology and Cancer Centre
Oxford University Hospitals Trust;
Professor of Medical Oncology
Sir William Dunn School of Pathology
University of Oxford
Oxford, United Kingdom
Chapter 217, Bone tumors

Lukas Haupt, MD
Second Department of Medicine
Hietzing Hospital
Vienna, Austria
Appendix: Classification and Diagnostic Criteria

Gillian A. Hawker, MD, MSc
Professor of Medicine
Division of Rheumatology
University of Toronto
Chief of Medicine
Women's College Hospital
Toronto, Ontario, Canada
Chapter 186, Assessment of the patient with osteoarthritis and measurement of outcomes

Philip N. Hawkins, PhD, FRCP, FRCPath, FMedSci
Professor of Medicine
University College London
National Amyloidosis Centre
Royal Free Hospital
London, United Kingdom
Chapter 177, Amyloidosis

Peter Heeringa, PhD
Professor of Pathology and Medical Biology
University of Groningen, University Medical Center Groningen
Groningen, The Netherlands
Chapter 162, Biology and immunopathogenesis of vasculitis

Turid Heiberg, PhD
Professor and Research Leader
Department of Research, Innovation and Education
Oslo University Hospital
Oslo, Norway
Chapter 104, Multidisciplinary non-pharmacologic approach to rheumatoid arthritis

Dick Heinegård, MD, PhD†
Professor, Department of Clinical Sciences
Division of Rheumatology–Molecular Skeletal Biology
Lund University Faculty of Medicine
Lund, Sweden
Chapter 2, The articular cartilage

†Deceased.

Simon M. Helfgott, MD, CM
Associate Professor of Medicine
Harvard Medical School;
Director of Education and Fellowship Training
Division of Rheumatology
Brigham and Women's Hospital
Boston, Massachusetts
Chapter 207, Rheumatoid manifestations of endocrine and lipid disease

Kim Henriksen, PhD
Head of Musculoskeletal Diseases
NordicBioscience
Herlev, Denmark
Chapter 198, Biochemical markers in bone disease

Rana S. Hinman, BPhysio, PhD
Professor of Physiotherapy
Centre for Health, Exercise, and Sports Medicine
University of Melbourne
Melbourne, Australia
Chapter 55, Principles of rehabilitation: physical and occupational therapy

Pauline Y.P. Ho, MBBCh (Honours), BSC, MSc, PhD, FRCP
Consultant Rheumatologist and Honorary Senior Lecturer
The Kellgren Centre for Rheumatology
Manchester Royal Infirmary
The University of Manchester
Manchester, United Kingdom
Chapter 128, Clinical features of psoriatic arthritis

Marc C. Hochberg, MD, MPH, MACP
Professor of Medicine and Epidemiology and Public Health
Head, Division of Rheumatology and Clinical Immunology
University of Maryland School of Medicine
Acting Director, Gerontology Research Education and Clinical Center
Veterans Affairs Maryland Health Care System
Baltimore, Maryland
Chapter 188, Management of osteoarthritis
Chapter 197, Pathophysiology of osteoporosis

Markus H. Hoffmann, PhD
Research Group Leader
Department of Medicine 3
Friedrich Alexander University Erlangen-Nuremberg
Erlangen, Germany
Chapter 99, Autoantibodies in rheumatoid arthritis

Michael F. Holick, MD, PhD
Professor of Medicine, Physiology and Biophysics
Section of Endocrinology, Nutrition, and Diabetes
Boston University School of Medicine
Boston, Massachusetts
Chapter 201, Osteomalacia and rickets

Christopher R. Holroyd, BM, MRCP
Consultant Rheumatologist
MRC Lifecourse Epidemiology Unit
University of Southampton
Southampton, United Kingdom
Chapter 195, Epidemiology and classification of osteoporosis

Cathy Holt, BEng, PhD
Professor of Biomechanics and Orthopaedic Engineering
School of Engineering
Cardiff University
Cardiff, Wales
Chapter 6, Biomechanics of peripheral Joints

Thomas W.J. Huizinga, MD, PhD
Professor of Rheumatology
Leiden University Medical Center
Leiden, The Netherlands
Chapter 71, Interleukin-6 inhibitors

M. Elaine Husni, MD, MPH
Staff Physician
Orthopedic and Rheumatologic Institute
Cleveland Clinic
Cleveland, Ohio
Chapter 127, Classification and epidemiology of psoriatic arthritis

Robert D. Inman, MD
Professor of Medicine and Immunology
University of Toronto Faculty of Medicine
Kremil Research Institute
University Health Network
Toronto, Ontario, Cananda
Chapter 118, Reactive arthritis

Dai Inoue, PhD
Assistant Professor of Radiology
Kanazawa University Hospital
Kanazawa, Japan
Chapter 178, IgG4-related disease

Zacharia Isaac, MD
Director, Interventional Physical Medicine and Rehabilitation
Department of Physical Medicine and Rehabilitation
Harvard Medical School
Medical Director, Comprehensive Spine Care Center
Brigham and Women's Hospital
Boston, Massachusetts
Chapter 81, Low back pain

John D. Isaacs, BSc (hon), MB BS, PhD
Professor of Clinical Rheumatology
Institute of Cellular Medicine
Newcastle University;
Consultant Rheumatologist
Musculoskeletal Directorate
Freeman Hospital
Newcastle upon Tyne, United Kingdom
Chapter 76, Emerging therapeutic targets

Maura D. Iversen, BSc, MPH, DPT, SD
Professor and Chair
Department of Physical Therapy
Northeastern University
Senior Instructor in Medicine
Harvard Medical School
Behavioral Scientist
Section of Clinical Sciences
Division of Rheumatology, Immunology & Allergy
Brigham & Women's Hospital
Boston, Massachusetts
Chapter 54, Arthritis patient education, self-management, and health promotion

Douglas A. Jabs, MD, MBA
Professor and Chair Emeritus of Ophthalmology
Professor of Medicine
Director, New York Eye and Ear Infirmary of Mount Sinai
The Mount Sinai Hospital
New York, New York;
Adjunct Professor of Epidemiology
The Johns Hopkins University
Bloomberg School of Public Health
Baltimore, Maryland
Chapter 36, The eyes in rheumatic disease

Sarada Jaimungal, MBBS
Fellow
Division of Endocrinology, Diabetes and Nutrition
University of Maryland School of Medicine
Baltimore, Maryland
Chapter 197, Pathophysiology of osteoporosis

Judith A. James, MD, PhD
Member and Chair
Arthritis and Clinical Immunology Research Program
Oklahoma Medical Research Foundation
Professor of Medicine and Adjunct Professor of Pathology
Oklahoma University Health Sciences Center
Oklahoma City, Oklahoma
Chapter 134, Preclinical features of systemic lupus erythematosus

M. Kassim Javaid, MBBS, BMedSCI, PhD, FRCP
Associate Professor
Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Science
University of Oxford
Oxford, United Kingdom
Chapter 215, Heritable connective tissue disorders

Pooya Javidan, MD
Fellow, Lower Extremity Reconstruction
Department of Orthopaedic Surgery
Scripps Green Hospital
La Jolla, California
Chapter 85, The hip

Rose-Marie Javier, MD
Senior Lecturer
Medical University Louis Pasteur;
Senior Attending Physician
Rheumatology Unit
University Hospital Hautpierre
Strasbourg, France
Chapter 211, Gaucher disease

Roy Jefferis, BSc, PhD, FRSC, CChem, MRCP, FRCPath, DSc
Professor Emeritus
School of Immunity and Infection
University of Birmingham
Birmingham, United Kingdom
Chapter 69, Biologic agents: monoclonal antibodies and receptor antagonists

Alyssa K. Johnsen, MD, PhD
Associate Director
Global Clinical Research
Bristol Myers Squibb
Princeton, New Jersey
Chapter 66, Methotrexate

Joanne M. Jordan, MD, MPH
Joseph P. Archie, Jr. Eminent Professor of Medicine
Vice Dean, Faculty Affairs and Leadership Development
University of North Carolina at Chapel Hill School of Medicine
Chapel Hill, North Carolina
Chapter 179, Epidemiology and classification of osteoarthritis

Andrew A. Joyce, MD
Resident Physician
Department of Physical Medicine and Rehabilitation
Harvard Medical School
Spaulding Rehabilitation Hospital
Boston, Massachusetts
Chapter 81, Low back pain

Michelle Jung, MD, FRCPC
Alberta Health Services
Calgary, Alberta, Canada
Chapter 142, Assessing disease activity and outcomes in systemic lupus erythematosus

Ruba Kado, MD
Clinical Instructor in Internal Medicine
Division of Rheumatology
University of Michigan Medical School
Ann Arbor, Michigan
Chapter 73, Interleukin-17, interleukin-12, and interleukin-23 inhibitors

Tsuneyasu Kaisho, MD, PhD
Professor
Department of Immunology
Institute of Advanced Medicine
Wakayama, Japan
Chapter 9, Principles of innate immunity

Cees G.M. Kallenberg, MD, PhD
Professor of Rheumatology and Clinical Immunology
University of Groningen, University Medical Center Groningen
Groningen, The Netherlands
Chapter 162, Biology and immunopathogenesis of vasculitis

David Kane, PhD, FRCPI
Clinical Professor of Rheumatology
Trinity College Dublin
Consultant Rheumatologist
Adelaide and Meath Hospital
Dublin, Ireland
Chapter 48, Musculoskeletal ultrasound

Mariana J. Kaplan, MD
Senior Investigator
Chief, Systemic Autoimmunity Branch
Intramural Research Program
National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institutes of Health
Bethesda, Maryland
Chapter 140, Pathogenesis of systemic lupus erythematosus

Morten Asser Karsdal, MSc, PhD, mMBA
Head of Research and Development
Nordic Bioscience
Herlev, Denmark
Chapter 198, Biochemical markers in bone disease

Dimitrios G. Kassimos, MD, MSc
Consultant Rheumatologist
Director of Education Department
401 General Military Hospital of Athens
Athens, Greece
Chapter 205, Neuropathic arthropathy

Daniel L. Kastner, MD, PhD
Scientific Director, Division of Intramural Research
National Human Genome Research Institute
National Institutes of Health
Bethesda, Maryland
Chapter 174, Monogenic autoinflammatory diseases

Mitsuhiro Kawano, MD, PhD
Director, Division of Rheumatology
Department of Internal Medicine
Kanazawa University Hospital
Kanazawa, Japan
Chapter 178, IgG4-related disease

Jonathan Kay, MD
Professor of Medicine
Division of Rheumatology
University of Massachusetts Medical School;
Director of Clinical Research
Division of Rheumatology
UMass Memorial Medical Center
Worcester, Massachusetts
Chapter 77, Biosimilars in rheumatology
Chapter 214, Miscellaneous arthropathies

Richard M. Keating, MD, MHS
Program Director, Rheumatology Fellowship
Division of Rheumatology
Scripps Clinic and Scripps Green Hospital
La Jolla, California
Chapter 67, Immunosuppressive agents: cyclosporine, cyclophosphamide, azathioprine, mycophenolate mofetil, and tacrolimus

Jennifer A. Kelly, MPH
Research Project Director
Arthritis and Clinical Immunology Research Program
Oklahoma Medical Research Foundation
Oklahoma City, Oklahoma
Chapter 136, Genetics of systemic lupus erythematosus

David Kendler, MD
Professor of Medicine
University of British Columbia
Vancouver, Canada
Chapter 51, Dual x-ray absorptiometry and measurement of bone

Munther A. Khamashta, MD, PhD, FRCP
Professor and Consultant Physician
King's College London;
Director, Graham Hughes Lupus Research Unit
St. Thomas Hospital
London, United Kingdom
Chapter 148, Antiphospholipid syndrome: pathogenesis, diagnosis, and management

Irfan R. Khan, MD
Assistant Professor of Ophthalmology
Johns Hopkins School of Medicine
Wilmer Eye Institute
Baltimore, Maryland
Chapter 36, The eyes in rheumatic disease

Dinesh Khanna, MD, MS
Professor of Medicine
Frederick G. l. Huetwell Professor of Rheumatology
Director, University of Michigan Scleroderma Program
Division of Rheumatology
University of Michigan Medical School
Ann Arbor, Michigan
Chapter 152, Assessing disease activity and outcome in systemic sclerosis

Mari Klokkerud, OT, PhD
Researcher
National Advisory Unit on Rehabilitation in Rheumatology
Diakonhjemmet Hospital
Department of Rheumatology
Oslo, Norway
Chapter 104, Multidisciplinary non-pharmacologic approach to rheumatoid arthritis

Kathleen D. Kolstad, MD, PhD
Fellow
Division of Immunology and Rheumatology
Stanford University School of Medicine
Stanford, California
Chapter 158, Clinical significance of autoantibodies in inflammatory muscle disease

Leah Kottyan, PhD
Research Associate
Center for Autoimmune Genomics and Etiology
Cincinnati Children's Hospital Medical Center
Cincinnati, Ohio
Chapter 136, Genetics of systemic lupus erythematosus

Virginia Byers Kraus, MD, PhD
Professor of Medicine (Rheumatology), Orthopaedics, and Pathology
Duke Molecular Physiology Institute
Duke University Medical Center
Durham, North Carolina
Chapter 187, Preclinical and early osteoarthritis
Chapter 211, Rare osteoarthritis: ochronosis and Kashin-Beck disease

Tore K. Kvien, MD, PhD
Professor of Rheumatology
University of Oslo Faculty of Medicine
Head, Department of Rheumatology
Diakonhjemmet Hospital
Olso, Norway
Chapter 104, Multidisciplinary non-pharmacologic approach to rheumatoid arthritis

Robert Lafyatis, MD
Professor of Medicine
Divison of Rheumatology
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania
Chapter 151, Etiology and pathogenesis of systemic sclerosis

Robert B.M. Landewé, MD
Professor of Clinical Immunology & Rheumatology
University of Amsterdam
Academic Medical Center
Amsterdam, The Netherlands;
Consultant Rheumatologist
Atrium Medical Center
Heerlen, The Netherlands
Chapter 29, Interpreting the medical literature for the rheumatologist
Chapter 50, Use of imaging as an outcome measure in clinical trials

Carol A. Langford, MD, MHS, FACP
Director, Center for Vasculitis Care and Research
Harold C. Schott Chair in Rheumatic and Immunologic Diseases
Cleveland Clinic;
Associate Professor of Medicine
Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Cleveland, Ohio
Chaper 165, Takayasu arteritis

Arthur N. Lau, MD, FRCPC
Assistant Professor
Division of Rheumatology
McMaster University
Michael G. DeGroote School of Medicine
Hamilton, Ontario, Canada
Chapter 200, Glucocorticoid-induced osteoporosis

Robert A. Lavin, MD, MS
Assistant Professor of Neurology
University of Maryland School of Medicine
Director of Chronic Pain Management
Department of Neurology
VA Maryland Health Care System
Adjunct Professor
Department of Occupational Medicine
Johns Hopkins University School of Medicine
Baltimore, Maryland
Chapter 62, Principles of pharmacologic pain management

Ronald M. Laxer, MDCM, FRCPC
Professor of Pediatrics and Medicine
Division of Rheumatology
University of Toronto Faculty of Medicine
The Hospital for Sick Children
Toronto, Ontario, Canada
Chapter 109, Management of juvenile idiopathic arthritis

Thomas J. Learch, MD
Chief of Musculoskeletal Imaging
Department of Imaging
Cedars-Sinai Medical Center
Los Angeles, California
Chapter 96, Imaging of rheumatoid arthritis

Cianna Leatherwood, MD
Rheumatology Fellow
Harvard Medical School
Brigham and Women's Hospital
Boston, Massachusetts
Chapter 59, Drugs and pregnancy
Chapter 207, Rheumatoid manifestations of endocrine and lipid disease

Jennifer S. Lewis, PhD MSc, Dip COT
Senior Clinical Research Occupational Therapist
Complex Regional Pain Syndrome and Cancer Late Effects Rehabilitation Service
Royal National Hospital for Rheumatic Diseases, Royal United Hospitals Bath NHS Trust
Bath, United Kingdom;
Senior Lecturer, Department of Allied Health Professions
University of the West of England
Bristol, United Kingdom
Chapter 56, Multi-disciplinary approaches to managing chronic pain in arthritis

George Lewith, MD, FRCP
Professor of Health Research
Primary Care and Population Sciences
University of Southampton Medical School
Honorary Consultant Physician
Primary Care and Population Sciences
Southampton University NHS Hospitals Trust
Southampton, United Kingdom
Chapter 58, Complementary and alternative medicine

Katherine P. Liao, MD, MPH
Assistant Professor of Medicine
Harvard Medical School
Associate Physician
Division of Rheumatology, Immunology, and Allergy
Brigham and Women's Hospital
Boston, Massachusetts
Chapter 92, Classification and epidemiology of rheumatoid arthritis

Adam P. Lightfoot, PhD
Lecturer
School of Healthcare Science
Manchester Metropolitan University
Manchester, United Kingdom
Chapter 42, The muscles in rheumatic disease

Geoffrey O. Littlejohn, MBBS(Hons), MD, MPH
Clinical Professor of Medicine
Monash University
Emeritus Director, Rheumatology Unit
Monash Medical Centre
Clayton, Victoria, Australia
Chapter 204, Diffuse idiopathic skeletal hyperostosis

Pilar Lorenzo, PhD
Researcher, Department of Clinical Sciences
Division of Rheumatology—Molecular Skeletal Biology
Lund University Faculty of Medicine
Lund, Sweden
Chapter 2, The articular cartilage

Rik J.U. Lories, MD, PhD
Professor of Medicine
Skeletal Biology and Engineering Research Center
Katholieke Universiteit Leuven
Leuven, Belgium
Chapter 130, Animal models of psoriatic arthritis

Thomas A. Luger, MD
Professor of Dermatology
University of Münster
Münster, Germany
Chapter 35, The skin in rheumatic disease

Ingrid E. Lundberg, MD, PhD
Professor of Rheumatology
Department of Medicine, Solna
Karolinska Institutet
Stockholm, Sweden
Chapter 157, Etiology and pathogenesis of inflammatory muscle disease (myositis)

Raashid A. Luqmani, DM, FRCP, FRCPE
Professor of Rheumatology
Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Science
University of Oxford;
Consultant Rheumatologist
Nuffield Orthopaedic Centre
Oxford, United Kingdom
Chapter 164, Antineutrophil cytoplasm antibody–associated vasculitis

Hanna M. Lythgoe, MBChB, MRCPCH
Clinical Research Fellow
Department of Women's and Children's Health, Institute of Translational Medicine
University of Liverpool
Clinical Research Fellow
NIHR Alder Hey Clinical Research Facility
Alder Hey Children's NHS Foundation Trust
Liverpool, United Kingdom
Chapter 111, Connective tissue diseases in children

Kristin J. MacDougall, MD
Division of Rheumatology
Columbia University College of Physicians and Surgeons
New York, New York
Chapter 103, Management of established rheumatoid arthritis

C. Ronald MacKenzie, MD
Attending Physician
Department of Medicine and Rheumatology
Hospital for Special Surgery
Professor of Clinical Medicine and Medical Ethics
Weill Medical College of Cornell University
C. Ronald MacKenzie Chair in Ethics and Medicine
Hospital for Special Surgery
New York, New York
Chapter 30, Ethics in clinical trials
Chapter 60, Perioperative care of the rheumatic disease patient

Julia Manasson, MD
Fellow
Division of Rheumatology
New York University School of Medicine
New York, New York
Chapter 24, The microbiome in rheumatic diseases

David Charles Mangham, BSc, MB BS, FRCPath
Professor of Applied Molecular Pathology
Deputy Director of MMPathIC
Faculty of Biology, Medicine and Health
University of Manchester
Manchester, United Kingdom
Chapter 34, Synovial fluid analysis

Rong Mao, PhD
Investigator
Department of Medicine
Stanford School of Medicine
Palo Alto, California
Chapter 14, The complement system

Lyn M. March, MBBS, MSc, PhD, FRACP, FAFPHM
Liggins Professor of Rheumatology and Musculoskeletal Epidemiology
University of Sydney Professorial Rheumatology Deparment
Royal North Shore Hospital
St Leonards, New South Wales, Australia
Chapter 53: Treatment recommendations and "treat to target"

Alejandro Olivé Marqués, MD, PhD
Rheumatology Service
Hospital Universitari Germans Trias i Pujol
Barcelona, Spain
Chapter 173, Adult-onset Still disease

Javier Márquez, MD
Rheumatologist
Department of Internal Medicine
Hospital Pablo Tobon Uribe
Medellín, Colombia
Chapter 114, Mycobacterial, brucellar, fungal, and parasitic arthritis

Emilio Martín-Mola, MD, PhD
Department of Rheumatology
Hospital Universitario La Paz
Madrid, Spain
Chapter 113, Bacterial arthritis

Manuel Martínez-Lavín, MD
Chief, Rheumatology Department
National Institute of Cardiology;
Professor of Rheumatology
National Autonomous University
Mexico City, Mexico
Chapter 213, Digital clubbing and hypertrophic osteoarthropathy

Eric L. Matteson, MD, MPH
Professor of Medicine
Divisions of Rheumatology and Epidemiology
Mayo Clinic College of Medicine
Rochester, Minnesota
Chapter 95, Extraarticular features of rheumatoid arthritis

Stephen J. Matzat, BA
Department of Radiology
Stanford School of Medicine
Palo Alto, California
Chapter 47, Functional magnetic resonance imaging

Maureen D. Mayes, MD, MPH
Professor of Medicine
Division of Rheumatology
University of Texas Health Science Center Houston
McGovern School of Medicine
Houston, Texas
Chapter 149, Classification and epidemiology of systemic sclerosis (scleroderma)

Timothy McAlindon, MD, MPH, MRCP
Professor of Medicine
Tufts University School of Medicine
Chief, Division of Rheumatology
Tufts Medical Center
Boston, Massachusetts
Chapter 206, Osteonecrosis

Candy S. McCabe, PhD, MSc
Florence Nightingale Foundation Chair in Clinical Nursing Practice Research
University of the West of England
Bristol, United Kingdom;
Royal United Hospitals NHS Foundation Trust
Royal National Hospital for Rheumatic Diseases
Bath, United Kingdom
Chapter 56, Multi-disciplinary approaches to managing chronic pain in arthritis

Edward F. McCarthy, MD
Professor of Pathology and Orthopaedic Surgery
Johns Hopkins University School of Medicine
Baltimore, Maryland
Chapter 217, Bone tumors

Geraldine M. McCarthy, MD, FRCPI
Clinical Professor of Medicine
School of Medicine and Medical Science
University College Dublin;
Consultant Rheumatologist
Mater Misericordiae University Hospital
Dublin, Ireland
Chapter 194, Basic calcium phosphate crystal deposition disease

Michael F. McDermott, MB, BCh, BAO, MRCPI, DMed
Professor of Experimental Rheumatology
University of Leeds—St James's University Hospital
National Institute of Health Research—Leeds Musculoskeletal Biomedical Research Unit
Leeds Institute of Rheumatic and Musculoskeletal Medicine
Leeds, United Kingdom
Chapter 174, Monogenic autoinflammatory diseases

Dennis McGonagle, PhD, FRCPI
Professor of Investigative Rheumatology
Leeds Institute of Rheumatic & Musculoskeletal Medicine
University of Leeds
Leeds, United Kingdom
Chapter 129, Etiology and pathogenesis of psoriatic arthritis

Lachy McLean, MD, PhD, FRCP
Vice President
Takeda Pharmaceuticals
San Diego, California
Chapter 190, Etiology and pathogenesis of gout

Andrew Robert Ian Melville, MBBS Lond, MA Camb, MRCP
Core Medical Trainee
Northwick Park Hospital
London, United Kingdom
Chapter 154, Raynaud phenomenon

Laëtitia Michou, MD, PhD
Associate Professor of Medicine
CHU de Québec-Université Laval
Endocrinology and Nephrology Unit
CHU de Québec Research Centre
Québec City, Canada
Chapter 203, Paget disease of bone

Rob Middleton, BMBCh, MA(Cantab), MRCS
Botnar Research Centre
Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Science
University of Oxford
Oxford, United Kingdom
Chapter 86, The knee

Jamal A. Mikdashi, MD, MPH
Associate Professor of Medicine
Division of Rheumatology and Clinical Immunology
University of Maryland School of Medicine
Baltimore, Maryland
Chapter 172, Primary angiitis of the central nervous system

Frederick W. Miller, MD, PhD
Chief, Environmental Autoimmunity Group
Program of Clinical Research
National Institute of Environmental Health Sciences
National Institutes of Health
Bethesda, Maryland
Chapter 159, Management of inflammatory muscle disease

Paul D. Miller, MD
Medical Director
Colorado Center for Bone Research
Lakewood, Colorado
Chapter 202, Renal osteodystrophy

Kirsten Minden, MD
Staff Scientist, Epidemiology Unit
German Rheumatism Research Center Berlin;
Consultant in Pediatric Rheumatology
Children´s University Hospital Charité
Berlin, Germany
Chapter 106, Classification and epidemiology of juvenile idiopathic arthritis

Jonathan J. Miner, MD, PhD
Instructor of Medicine
Division of Rheumatology
Washington Unviersity School of Medicine in St. Louis
St. Louis, Missouri
Chapter 94, Clinical features of rheumatoid arthritis

Rikke Helene Moe, PhD, PT, MSc
Researcher
National Advisory Unit on Rehabilitation in Rheumatology
Diakonhjemmet Hospital
Department of Rheumatology
Oslo, Norway
Chapter 104, Multidisciplinary non-pharmacologic approach to rheumatoid arthritis

Ingrid Möller, MD, PhD
Assistent Professor of Anatomy
University of Barcelona
Director, Department of Rheumatology
Instituto Poal de Reumatología
Barcelona, Spain
Chapter 44, Aspiration and injection of joints and periarticular tissues and intralesional therapy

Paul A. Monach, MD, PhD
Chief, Rheumatology Section
VA Boston Healthcare System
Associate Professor, Section of Rheumatology
Boston University School of Medicine
Chapter 100, Pathogenesis and pathology of rheumatoid arthritis

Barend Mons, PhD
Professor in Bio-Semantics
Department of Human Genetics
Leiden University Medical Centre
Leiden, The Netherlands
Chapter 20, Open Science for Systems biology

Elena Myasoedova, MD, PhD
Assistant Professor of Medicine
Mayo Clinic College of Medicine
Rochester, Minnesota
Chapter 95, Extraarticular features of rheumatoid arthritis

Jackie L. Nam, MBBCh, FCP, Rheum cert, PhD
Rheumatology Clinical Research Fellow
Leeds Institute of Rheumatic and Musculoskeletal Medicine
University of Leeds
Leeds, United Kingdom
Chapter 102, Management of early rheumatoid arthritis

Gauthier Namur, MD
Department of Nuclear Medicine
Christian Hospital Center
Liège, Belgium
Chapter 49, Bone scintigraphy and positron emission tomography

Esperanza Naredo, MD
Department of Rheumatology
Hospital General Universitario Gregorio Marañon
Madrid, Spain
Chapter 44, Aspiration and injection of joints and periarticular tissues and intralesional therapy

Barbara Neerinckx, MD
Skeletal Biology and Engineering Research Center
Katholieke Universiteit Leuven
Leuven, Belgium
Chapter 130, Animal models of psoriatic arthritis

Amanda E. Nelson, MD, MSCR
Assistant Professor of Medicine
Division of Rheumatology, Allergy, and Immunology
Thurston Arthritis Research Center
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina
Chapter 179, Epidemiology and classification of osteoarthritis

Philippa J.A. Nicolson, BPhty
Doctoral Candidate
Centre for Health, Exercise and Sports Medicine
University of Melbourne
Melbourne, Australia
Chapter 55, Principles of rehabilitation: physical and occupational therapy

César E. Fors Nieves, MD
Instructor, Department of Medicine
Division of Rheumatology
New York University School of Medicine
Associate Director
Rheumatology Fellowship Program
NYU Medical Center
Hospital for Joint Diseases
New York, New York
Chapter 79, Precision medicine and pharmacogenomics in rheumatology

Ellen B. Nordal, MD, PhD
Consultant, Department of Pediatrics
University Hospital of Northern Norway
Associate Professor, Department of Clinical Medicine
UIT the Arctic University of Norway
Tromsø, Norway
Chapter 107, Clinical features of juvenile idiopathic arthritis

Ulrich Nöth, MD, MHBA
Senior Consultant and Chief
Department of Orthopedics and Trauma Surgery
Evangelisches Waldkrankenhaus Spandau
Berlin, Germany
Chapter 18, Principles of tissue engineering and cell- and gene-based therapy

Eleana Ntatsaki, MRCP(Rheumatology)(UK), MA MedEd, FHEA
Consultant in Rheumatology
Ipswich Hospital NHS Trust
Ipswich, United Kingdom;
Honorary Senior Clinical Lecturer
University College London Medical School
London, United Kingdom
Chapter 161, Classification and epidemiology of vasculitis

Chester V. Oddis, MD
Professor of Medicine
Division of Rheumatology and Clinical Immunology
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania
Chapter 156, Classification, epidemiology, and clinical features of inflammatory muscle disease

Ahmed Omar, MBBch, MRCP
Clinical Fellow
Division of Rheumatology
University of Toronto Faculty of Medicine
Toronto, Ontario, Canada
Chapter 118, Reactive arthritis

Michael J. Ombrello, MD
Investigator, Translational Genetics and Genomics Unit
National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institutes of Health
Bethesda, Maryland
Chapter 19, Principles and techniques in molecular biology

Antonina Omisade, PhD
Psychologist
Queen Elizabeth II Health Sciences Centre
Halifax, Nova Scotia, Canada
Chapter 144, Management of central nervous system lupus

Voon H. Ong, PhD, MRCP
Senior Lecturer in Rheumatology
Centre for Rheumatology
University College London
Royal Free Hospital
London, United Kingdom
Chapter 150, Clinical and serologic features of systemic sclerosis

Patrik Önnerfjord, PhD
Associate Professor, Department of Clinical Sciences
Division of Rheumatology—Molecular Skeletal Biology
Lund University Faculty of Medicine
Lund, Sweden
Chapter 2, The articular cartilage

Philippe Orcel, MD, PhD
Professor of Rheumatology
Paris-Diderot Faculty of Medicine
University of Paris 7
Chief, Department of Musculoskeletal Diseases
Rheumatology and Bone Diseases
Hospital Lariboisière, Assistance Publique Hôpitaux de Paris
Paris, France
Chapter 211, Gaucher disease

Carl Orr, MB BAO, BCh, BMedSci, MSc LMD, MRCPI, PhD
The Centre for Arthritis and Rheumatic Disease
University College Dublin
Dublin, Ireland
Chapter 131, Management of psoriatic arthritis

John J. O'Shea, MD
Scientific Director
National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institutes of Health
Bethesda, Maryland
Chapter 11, Signal transduction in immune cells
Chapter 12, Cytokines

Caroline Ospelt, MD, PhD
Senior Researcher
Center of Experimental Rheumatology
University Hospital Zürich
Zürich, Switzerland
Chapter 23, Epigenetics

Seza Özen, MD
Professor of Pediatric Rheumatology
Hacettepe University Faculty of Medicine
Ankara, Turkey
Chapter 169, IgA vasculitis (Henoch-Schoenlein purpura)

Matthew J.S. Parker, MBChB, MRCP
Advanced Trainee
Department of Rheumatology
Royal Prince Alfred Hospital
Sydney, Australia
Chapter 208, Hemophilia and von Willebrand disease

Carlo Patrono, MD
Professor of Pharmacology
Catholic University School of Medicine
Rome, Italy
Chapter 63, Nonsteroidal antiinflammatory drugs

Clarissa A. Pilkington, MBBS, BSc, FRCP, FRCPCH
Honorary Lecturer
Institute of Child Health
University College London Medical School
Consultant in Paediatric and Adolescent Rheumatology
Great Ormond Street Hospital NHS Trust
London, United Kingdom
Chapter 111, Connective tissue diseases in children

Michael H. Pillinger, MD
Associate Professor of Medicine and Pharmacology
Director, Rheumatology Training
Director, Masters of Science in Clinical Investigation Program
New York University School of Medicine
Section Chief, Rheumatology
New York Harbor Health Care System–NY Campus
Department of Veterans Affairs
New York, New York
Chapter 13, Inflammation and its chemical mediators

Carlos Pineda, MD, MSc
Research Director
Instituto Nacional de Rehabilitación
Mexico City, Mexico
Chapter 213, Digital clubbing and hypertrophic osteoarthropathy

Nicolò Pipitone, MD, PhD
Unità di Reumatologia
Azienda Ospedaliera IRCCS di Reggio Emilia
Reggio Emilia, Italy;
Dipartimento Biomedico di Medicina Interna a Specialistica
Sezione di Reumatologia
Università degli Studi di Palermo
Palermo, Italy
Chapter 166, Polymyalgia rheumatica and giant cell arteritis

Denis Poddubnyy, MD
Professor of Rheumatology
Head, Rheumatology Unit
Charité University Hospital
Berlin, Germany
Chapter 126, Management of axial spondyloarthritis

Janet E. Pope, MD, MPH, FRCPC
Professor of Medicine
University of Western Ontario
Head, Division of Rheumatology
St. Joseph's Health Care
London, Ontario, Canada
Chapter 142, Assessing disease activity and outcomes in systemic lupus erythematosus

Anna Postolova, MD, MPH
Fellow
Division of Immunology and Rheumatology
Stanford University School of Medicine
Stanford, California
Chapter 158, Clinical significance of autoantibodies in inflammatory muscle disease

Andrew Price, MBBS, BSc, Dphil, FRCS
Professor of Orthopaedic Surgery
Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Science
University of Oxford
Consultant Orthopaedic Surgeon
Nuffield Orthopaedic Centre
Oxford University Hospitals Trust
Oxford, United Kingdom
Chapter 86, The knee

Luca Quartuccio, MD, PhD
Rheumatology Clinic
Department of Medical Area
Academic Hospital 'S. Maria della Misericordia'
Udine, Italy
Chapter 171, Cryoglobulinemia

Lars Rackwitz, MD
Senior Consultant
Department of Orthopaedics and Trauma Surgery
Evangelisches Waldkrankenhaus Spandau
Berlin, Germany
Chapter 18, Principles of tissue engineering and cell- and gene-based therapy

Helga Radner, MD
Medical University Vienna
Department of Internal Medicine III
Division of Rheumatology
Vienna, Austria
Chapter 43, Multimorbidity

Soumya Raychaudhuri, MD, PhD
Associate Professor of Medicine
Harvard Medical School
Associate Physician
Divisions of Rheumatology and Genetics
Brigham and Women's Hospital
Boston, Massachusetts;
Professor
Arthritis Research UK Centre for Genetics and Genomics
University of Mancehster
Manchester, United Kingdom
Chapter 97, Genetics of rheumatoid arthritis

Anthony C. Redmond, PhD
Professor and Head
Section of Clinical Biomechanics and Physical Medicine
Leeds Institute of Rheumatic and Musculoskeletal Medicine
University of Leeds;
NIHR Leeds Biomedical Research Unit
Leeds Teaching Hospitals NHS Trust
Leeds, United Kingdom
Chapter 87, The ankle and foot

Jonathan L. Rees, MBBS, FRCS (Eng), MD, FRCS (Tr & Orth)
Professor
Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Science
University of Oxford
Oxford, United Kingdom
Chapter 82, The shoulder

Westley H. Reeves, MD
Marcia Whitney Schott Professor of Medicine
Division of Rheumatology and Clinical Immunology
University of Florida College of Medicine
Gainesville, Florida
Chapter 139, Autoantibodies in systemic lupus erythematosus

Linda M. Rehaume, PhD
Research Fellow
Translational Research Institute
The University of Queensland Diamantina Institute
Brisbane, Australia
Chapter 124, Animal models of spondyloarthritis

Elaine F. Remmers, PhD
Associate Investigator, Inflammatory Disease Section
National Human Genome Research Institute
National Institutes of Health
Bethesda, Maryland
Chapter 19, Principles and techniques in molecular biology

Luis Requena, MD
Professor of Dermatology
Universidad Autónoma
Chairman, Department of Dermatology
Fundación Jiménez Diaz
Madrid, Spain
Chapter 170, Cutaneous vasculitis and panniculitis

Gary Reynolds, BSc, MBBS, PhD
Academic Clinical Lecturer
Musculoskeletal Research Group
Newcastle University
Newcastle upon Tyne, United Kingdom
Chapter 76, Emerging therapeutic targets

Clio Ribbens, MD, PhD
Head of Clinic
Department of Rheumatology
University Hospital of Liège
Liège, Belgium
Chapter 49, Bone scintigraphy and positron emission tomography

Bethan Richards, MBBS, MClinEpi, M Sports Med
Clinical Senior Lecturer in Medicine
University of Sydney;
Head, Department of Rheumatology
Royal Prince Alfred Hospital
Sydney, New South Wales, Australia
Chapter 53: Treatment recommendations and "treat to target"

Bruce C. Richardson, MD, PhD
Professor of Internal Medicine
University of Michigan Medical School
Chief, Section of Rheumatology
Veterans Affairs Hospital
Ann Arbor, Michigan
Chapter 141, Drug-induced lupus

Pascal Richette, MD, PhD
Professor of Rheumatology
Université Paris Diderot
Assistance Publique-Hôpitaux de Paris
Hôpital Lariboisière
Paris, France
Chapter 210, Hemochromatosis

Adrian Richter, PhD, MSc
Epidemiology Unit
German Rheumatism Research Centre
Berlin, Germany
Chapter 31, European biologics registers

Christopher T. Ritchlin, MD, MPH
Professor of Medicine
Center for Musculoskeletal Research
University of Rochester Medical Center
Rochester, New York
Chapter 129, Etiology and pathogenesis of psoriatic arthritis

Susan Y. Ritter, MD, PhD
Associate Physician
Division of Rheumatology, Allergy and Immunology
Brigham and Women's Hospital;
Instructor in Medicine
Harvard Medical School
Boston, Massachusetts
Chapter 176, Relapsing polychondritis

William H. Robinson, MD, PhD
Associate Professor of Medicine
Division of Immunology and Rheumatology
Stanford School of Medicine
Palo Alto, California
Chapter 14, The complement system

Valerie Rogers, MBBS, MRCPCH, BA (Hons) QTS
Department of Paediatric Rheumatology
Bristol Royal Hospital for Children
Bristol, United Kingdom;
Consultant Paediatrician
Bath Centre for Pain Services
Royal National Hospital For Rheumatic Diseases
Bath, United Kingdom
Chapter 216, Hypermobility syndrome

Ivan O. Rosas, MD
Associate Professor of Medicine
Harvard Medical School
Pulmonary and Critical Care Division
Brigham and Women's Hospital
Boston, Massachusetts
Chapter 38, The lung in rheumatic disease

Ann K. Rosenthal, MD
Will and Cava Ross Professor of Medicine
Chief of Rheumatology
Medical College of Wisconsin;
Staff Physician
Clement J. Zablocki VA Medical Center
Milwaukee, Wisconsin
Chapter 193, Calcium pyrophosphate deposition disease (pseudogout)

Andrew D. Rowan, PhD
Professor of Molecular Rheumatology
Head of the Musculoskeletal Research Group
Institute of Cellular Medicine
Newcastle University
Newcastle upon Tyne, United Kingdom
Chapter 17, Tissue destruction and repair

Martin Rudwaleit, MD
Professor of Internal Medicine and Rheumatology
Klinikum Bielefeld
Bielefeld, Germany;
Professor
Charité University Medicine
Berlin, Germany;
Professor of Rheumatology
University Hospital Ghent
Ghent, Belgium
Chapter 119, Classification and epidemiology of spondyloarthritis

Marina Rull, MD
Chief, Immunology and Rheumatology Department
Instituto Nacional de Ciencias Medicas y Nutrición, Salvador Zubirán
México City, México
Chapter 44, Aspiration and injection of joints and periarticular tissues and intralesional therapy

Marite Rygg, MD, PhD
Professor, Department of Laboratory Medicine, Children's and Women's Health
Norwegian University of Science and Technology
Consultant, Department of Pediatrics
St. Olav's Hospital
Trondheim, Norway
Chapter 107, Clinical features of juvenile idiopathic arthritis

Kenneth G. Saag, MD, MSc
Jane Knight Lowe Professor of Medicine
Division of Clinical Immunology and Rheumatology, Department of Medicine
University of Alabama at Birmingham
Birmingham, Alabama
Chapter 64, Systemic glucocorticoids in rheumatology

Jane E. Salmon, MD
Professor of Medicine
Weill Cornell Medical College
Collette Kean Research Chair
Hospital for Special Surgery
New York, New York
Chapter 137, Immunopathology of systemic lupus erythematosus

Donald M. Salter, MBChB, MD
Professor of Osteoarticular Pathology
Centre for Genomics and Experimental Medicine
University of Edinburgh
MRC Institute of Genetics and Molecular Medicine
Edinburgh, United Kingdom
Chapter 5, Connective tissue responses to mechanical stress
Chapter 183, Pathogenesis and pathology of osteoarthritis

Carlo Salvarani, MD
Director, Division of Rheumatology
Azienda Ospedaliera IRCCS di Reggio Emilia
Reggio Emilia, Italy;
Dipartimento Biomedico di Medicina Interna a Specialistica
Sezione di Reumatologia
Università degli Studi di Palermo
Palermo, Italy
Chapter 166, Polymyalgia rheumatica and giant cell arteritis

Linda J. Sandell, PhD
Mildred B. Simon Research Professor and Director of Research
Director, Center for Musculoskeletal Research
Department of Orthopaedic Surgery
Professor, Departments of Cell Biology and Physiology and Biomedical Engineering
Washington University School of Medicine in St. Louis
St. Louis, Missouri
Chapter 2, The articular cartilage

Sabina Sandigursky, MD, MS
Department of Rheumatology
New York University School of Medicine
New York, New York
Chapter 13, Inflammation and its chemical mediators

Tore Saxne, MD, PhD
Professor, Department of Clinical Sciences
Division of Rheumatology—Molecular Skeletal Biology
Lund University Faculty of Medicine
Lund, Sweden
Chapter 2, The articular cartilage

Prof. Dr. Hans-Georg Schaible
Professor of Physiology
Friedrich Schiller University Jena
Director, Institute of Physiology
Jena University Hospital
Jena, Germany
Chapter 8, Scientific basis of pain

Jose U. Scher, MD
Assistant Professor of Medicine
Division of Rheumatology
New York University School of Medicine
Director, Arthritis Clinic
NYU-Hospital for Joint Diseases
New York, New York
Chapter 24, The microbiome in rheumatic diseases

Georg Schett, MD
Professor of Rheumatology and Immunology
Department of Internal Medicine 3
University of Erlangen-Nuremberg Faculty of Medicine
Erlangen, Germany
Chapter 16, Osteoimmunology

Adam I. Schiffenbauer, MD
Staff Clinician
Environmental Autoimmunity Group
National Institute of Environmental Health Sciences
National Institutes of Health
Bethesda, Maryland
Chapter 159, Management of inflammatory muscle disease

Alan L. Schiller, MD
Founding Chair of Pathology
Nova Southeastern University Medical School;
Director, Pathology Residency Program
HCA Hospitals
Miami, Florida
Chapter 217, Bone tumors

Naomi Schlesinger, MD
Professor of Medicine
Chief, Division of Rheumatology
Rutgers Robert Wood Johnson Medical School
New Brunswick, New Jersey
Chapter 191, Clinical features of gout

Benjamin E. Schreiber, MBBS, MA, MRCP
Consultant in Pulmonary Hypertension and Rheumatology
Royal Free Hospital
Honorary Senior Lecturer
Department of Medicine
University College London
London, United Kingdom
Chapter 37, The heart in rheumatic disease

Daniella Muallem Schwartz, MD
Clinical Fellow
Molecular Immunology and Inflammation Branch, Translational Immunology Section
National Institute of Arthritis Musculoskeletal and Skin Disease
National Institutes of Health
Bethesda, Maryland
Chapter 11, Signal transduction in immune cells

Lauren M. Shapiro, BA
Department of Radiology
Stanford School of Medicine
Palo Alto, California
Chapter 47, Functional magnetic resonance imaging

Leena Sharma, MD
Professor of Medicine
Northwestern University Feinberg School of Medicine
Chicago, Illinois
Chapter 180, Local and systemic risk factors for incidence and progression of osteoarthritis

Bernadette C. Siaton, MD
Assistant Professor of Medicine
Division of Rheumatology and Clinical Immunology
University of Maryland School of Medicine
Baltimore, Maryland
Chapter 89, Entrapment neuropathies and compartment syndromes

Richard M. Siegel, MD, PhD
Chief, Immunoregulation Section
Autoimmunity Branch
National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institutes of Health
Bethesda, Maryland
Chapter 12, Cytokines

Joachim Sieper, PhD
Professor of Rheumatology
Charité University Hospital
Berlin, Germany
Chapter 126, Management of axial spondyloarthritis

Julia Fridman Simard, ScD
Assistant Professor
Departments of Health Research and Policy (Epidemiology) and Medicine (Immunology and Rheumatology)
Stanford University School of Medicine
Stanford, California;
Assistant Professor of Medicine
Clinical Epidemiology Unit
Karolinska Institutet
Stockholm, Sweden
Chapter 25, Principles of epidemiology

Barry P. Simmons, MD
Associate Professor of Orthopaedic Surgery
Harvard Medical School
Chief, Hand and Upper Extremity Service
Brigham and Women's Hospital
Boston, Massachusetts
Chapter 84, The wrist and hand

Robert W. Simms, MD
Professor of Medicine
Boston University School of Medicine
Chief, Section of Rheumatology
Boston Medical Center
Boston, Massachusetts
Chapter 155, Localized scleroderma and scleroderma-like syndromes

Nora G. Singer, MD
Professor of Medicine and Pediatrics
Case Western Reserve University School of Medicine
Director, Division of Rheumatology
MetroHealth Medical Center
Cleveland, Ohio
Chapter 105, Evaluation of the child with rheumatologic complaints

Judith A. Smith, MD, PhD
Associate Professor of Pediatrics
Division of Allergy, Immunology, and Rheumatology
University of Wisconsin School of Medicine and Public Health
Madison, Wisconsin
Chapter 110, The juvenile-onset spondyloarthropathies

Stacy E. Smith, MD
Chief and Distinguished Barbara N. Weissman Chair
Division of Musculoskeletal Imaging and Intervention
Brigham and Women's Hospital;
Assistant Professor of Radiology
Harvard Medical School
Boston, Massachusetts
Chapter 46, Conventional radiography and computed tomography

Josef S. Smolen, MD, FRCP
Professor of Internal Medicine
Chair, Division of Rheumatology
Medical University of Vienna;
Chairman, Center for Rheumatic Diseases
Hietzing Hospital
Vienna, Austria
Chapter 101, Assessment of the patient with rheumatoid arthritis and the measurement of outcomes

Daniel H. Solomon, MD, MPH
Professor of Medicine
Harvard Medical School
Brigham and Women's Hospital
Boston, Massachusetts
Chapter 43, Multimorbidity

John Stack, MB, BCh, BAO
Specialist Registrar in Rheumatology
Mater Misericordiae University Hospital
Dublin, Ireland
Chapter 194, Basic calcium phosphate crystal deposition disease

David Stanley, MB BS, BSc (Hons), FRCS
Consultant Orthopaedic Surgeon
BMI Thornbury Hospital
Sheffield, United Kingdom
Chapter 83, The elbow

Virginia D. Steen, MD
Professor of Medicine
Division of Rheumatology, Clinical Immunology, and Allergy
Georgetown University School of Medicine
Washington, D.C.
Chapter 153, Management of systemic sclerosis

Allen C. Steere, MD
Professor of Medicine
Harvard Medical School
Director, Translational Research in Rheumatology
Massachusetts General Hospital
Boston, Massachusetts
Chapter 116, Lyme and other tick-borne diseases

Günter Steiner, PhD
Head of Research Lab
Division of Rheumatology
Medical University of Vienna
Coordinator
Ludwig Boltzmann Cluster for Arthritis and Rehabilitation
Vienna, Austria
Chapter 99, Autoantibodies in rheumatoid arthritis

Andre F. Steinert, MD
Associate Professor
Department of Orthopedic Surgery
Julius-Maximilians-University
Würzburg, Bavaria, Germany
Head of Department
Department of Orthopedic and Trauma Surgery
Agatharied Hospital
Haushab, Bavaria, Germany
Chapter 18, Principles of tissue engineering and cell- and gene-based therapy

George Stojan, MD
Instructor in Medicine
Division of Rheumatology
Harvard Medical School
Beth Israel Deaconess Medical Center
Boston, Massachusetts
Chapter 160, Metabolic, drug-induced, and other noninflammatory myopathies

Vibeke Strand, MD, MACR, FACP
Adjunct Clinical Professor of Medicine
Division of Immunology/Rheumatology
Stanford University School of Medicine
Palo Alto, California
Chapter 142, Assessing disease activity and outcomes in systemic lupus erythematosus

Rainer H. Straub, MD
Professor of Internal Medicine
University Hospital
Regensburg, Germany
Chapter 15, Neuroendocrine immune crosstalk in chronic inflammatory systemic diseases

Elizabeth A. Streeten, MD
Associate Professor of Medicine
Division of Endocrinology, Diabetes & Nutrition
University of Maryland School of Medicine
Baltimore, Maryland
Chapter 197, Pathophysiology of osteoporosis

Giulio Superti-Furga, PhD
CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences
Vienna, Austria
Chapter 22, Proteomics

Paul P. Tak, MD, PhD
Professor
Department of Clinical Immunology & Rheumatology
Academic Medical Center, University of Amsterdam
Amsterdam, The Netherlands;
Chief Immunology Officer and Senior Vice President R&D Pipeline
Department of Research and Development
GlaxoSmithKline
Cambridge, United Kingdom
Chapter 45, Minimally invasive procedures

Chen Tang, MD
Department of Internal Medicine
Cedars-Sinai Medical Center
Los Angeles, California
Chapter 96, Imaging of rheumatoid arthritis

Peter C. Taylor, MA, PhD, FRCP
Professor of Rheumatology
Kennedy Institute of Rheumatology
Nuffield Institute of Orthopedics, Rheumatology, and Musculoskeletal Sciences
University of Oxford
Botnar Research Centre
Headington, Oxford
Chapter 72, Tumor necrosis factor inhibitors

Robert Terkeltaub, MD
Chief, Rheumatology Section
VA Healthcare System
San Diego, California;
Professor of Medicine
Division of Rheumatology, Allergy, and Immunology
University of California, San Diego
La Jolla, California
Chapter 192, Management of gout and hyperuricemia

Beje Thomas, MD
Assistant Professor of Medicine
Division of Nephrology
University of Maryland School of Medicine
Baltimore, Maryland
Chapter 40, The kidneys in rheumatic disease

Ranjeny Thomas, MBBS, MD
Professor and Chair
Department of Rheumatology
Translational Research Institute
The University of Queensland Diamantina Institute
Brisbane, Australia
Chapter 124, Animal models of spondyloarthritis

Jennifer E. Thorne, MD, PhD
Associate Professor of Ophthalmology
Johns Hopkins University School of Medicine
Associate Professor of Epidemiology
Center for Clinical Trials
Johns Hopkins University Bloomberg School of Public Health
Baltimore, Maryland
Chapter 36, The eyes in rheumatic disease

Jonathan H. Tobias, MD, PhD
Professor of Rheumatology
Musculoskeletal Research Unit
Bristol Medical School
Bristol, United Kingdom
Chapter 196, Clinical features of osteoporosis

Adriana H. Tremoulet, MD, MAS
Associate Professor of Pediatrics
Associate Director, Kawasaki Disease Research Center
University of California San Diego School of Medicine
La Jolla, California
Chapter 168, Kawasaki disease

Leendert A. Trouw, PhD
Department of Rheumatology
Leiden University Medical Center
Leiden, The Netherlands
Chapter 99, Autoantibodies in rheumatoid arthritis

Rocky S. Tuan, PhD
Distinguished Professor and Director
Department of Orthopaedic Surgery
Center for Cellular and Molecular Engineering
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania
Chapter 18, Principles of tissue engineering and cell- and gene-based therapy

Carl Turesson, MD, PhD
Professor of Rheumatology
Skåne University Hospital
Lund University
Malmö, Sweden
Chapter 95, Extraarticular features of rheumatoid arthritis

Hisanori Umehara, MD, PhD
Director of Rheumatology and Immunology
Nagahama City Hospital
Shiga, Japan
Chapter 178, IgG4-related disease

Ana M. Valdes, MA, PhD
Associate Professor and Reader in Musculoskeletal Genetics
Academic Rheumatology
University of Nottingham
Nottingham, United Kingdom
Chapter 184, Genetics of osteoarthritis

Désirée van der Heijde, MD, PhD
Professor of Rheumatology
Leiden University Medical Center
Leiden, The Netherlands
Chapter 50, Use of imaging as an outcome measure in clinical trials

Ronald F. van Vollenhoven, MD, PhD
Professor and Chief, Clinical Therapy Research
Inflammatory Diseases (ClinTRID)
The Karolinska Institute
Senior physician
Chief, Clinical Trials Unit
Division of Rheumatology
The Karolinska University Hospital
Stockholm, Sweden
Chapter 68: Tyrosine kinase inhibition

Joke Vanderstukken, MD
Department of Immunology Allergology and Rheumatology
University of Antwerp, Faculty of Medicine and Health Sciences
Antwerp, Belgium;
Professor of Rheumatology
Leiden University Medical Center
Leiden, The Netherlands
Chapter 71, Interleukin-6 inhibitors

John Varga, MD
John and Nancy Hughes Professor
Department of Medicine
Northwestern University Feinberg School of Medicine
Chicago, Illinois
Chapter 151, Etiology and pathogenesis of systemic sclerosis

Dimitrios Vassilopoulos, MD
Professor of Medicine (Rheumatology)
Joint Rheumatology Program
National and Kapodistrian University of Athens School of Medicine
2nd Department of Medicine and Laboratory
Hippokration General Hospital
Athens, Greece
Chapter 115, Viral infections

Douglas J. Veale, MD, FRCPI, FRCP(Lon)
Director of Translational Research
The Centre for Arthritis and Rheumatic Disease;
Professor of Medicine
University College Dublin;
Fellow, Conway Institute of Biomolecular and Biomedical Medicine
Dublin, Ireland
Chapter 131, Management of psoriatic arthritis

Sebastien Viatte, MD, PhD
Lecturer in Genetics
Arthritis Research UK Centre for Genetics and Genomics
Centre for Musculoskeletal Research
Associate Member
Manchester Collaborative Centre for Inflammation Research (MCCIR)
Faculty of Biology, Medicine and Health
The University of Manchester
Manchester, United Kingdom
Chapter 97, Genetics of rheumatoid arthritis

Tonia Vincent, MBBS, PhD, FRCP
Professor of Musculoskeletal Biology
Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Sciences
Kennedy Institute of Rheumatology;
Honorary Consultant Rheumatologist
Oxford University Hospitals Trust
Oxford, United Kingdom
Chapter 182, Animal models of osteoarthritis

Edward M. Vital, MB ChB, MRCP, PhD
Associate Professor and Honorary Consultant
Leeds Institute of Rheumatic and Musculoskeletal Medicine
University of Leeds
NIHR Leeds Musculoskeletal Biomedical Research Centre
Leeds Teaching Hospitals NHS Trust
Leeds, United Kingdom
Chapter 75, Inhibitors of B cells

Isabell S. von Loga, AB, MBBS
Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Sciences
Kennedy Institute of Rheumatology
University of Oxford
Oxford, United Kingdom
Chapter 182, Animal models of osteoarthritis

Daniel J. Wallace, MD
Professor of Medicine
Division of Rheumatology
Cedars-Sinai Medical Center;
Clinical Professor of Medicine
Division of Rheumatology
David Geffen School of Medicine at UCLA
Los Angeles, California
Chapter 135, Clinical features of systemic lupus erythematosus

Nicola Walsh, PhD, MSc, MCSP
Professor of Knowledge Mobilisation and Musculoskeletal Health
Centre for Health and Clinical Research
University of the West of England
Bristol, United Kingdom
Chapter 56, Multi-disciplinary approaches to managing chronic pain in arthritis

Sara L. Warber, MD
Professor Emerita of Family Medicine
Former Director, Integrative Medicine
University of Michigan Medical School
Ann Arbor, Michigan
Chapter 57, Placebos, caring, and healing in rheumatology

Gary Warburton, DDS, MD, FDSRCS, FACS
Associate Professor of Oral and Maxillofacial Surgery
University of Maryland School of Medicine,
Baltimore, Maryland
Chapter 88, The temporomandibular joint

Robert J. Ward, MD, CCD
Assistant Professor of Radiology
Chief, Division of Musculoskeletal Imaging
Tufts Medical Center
Boston, Massachusetts
Chapter 206, Osteonecrosis

Laura Watts, MA (Oxon), BMBCh, MRCP
Rheumatology Registrar
Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Science
University of Oxford
Oxford, United Kingdom
Chapter 215, Heritable connective tissue disorders

Richard A. Watts, MA, DM, FRCP
Consultant Rheumatologist
Ipswich Hospital NHS Trust
Ipswich, United Kingdom;
Clinical Senior Lecturer
Norwich Medical School
University of East Anglia
Norwich, United Kingdom
Chapter 161, Classification and epidemiology of vasculitis

Lucy R. Wedderburn, MD, MA, PhD, FRCP
Professor in Paediatric Rheumatology
Institute of Child Health
University College London Medical School
Consultant in Paediatric Rheumatology
Great Ormond Street Hospital NHS Trust
London, United Kingdom
Chapter 111, Connective tissue diseases in children

Michael E. Weinblatt, MD
John R. and Eileen K. Riedman Professor of Medicine
Harvard Medical School
Division of Rheumatology, Immunology and Allergy
Brigham and Women's Hospital
Boston, Massachusetts
Chapter 66, Methotrexate

Matthew R. Weir, Sr., MD
Professor of Medicine
Director, Division of Nephrology
University of Maryland School of Medicine
Baltimore, Maryland
Chapter 40, The kidneys in rheumatic disease

Michael H. Weisman, MD
Cedars-Sinai Chair in Rheumatology
Director, Division of Rheumatology
Professor of Medicine
Cedars-Sinai Medical Center
Distinguished Professor of Medicine
David Geffen School of Medicine at University of California, Los Angeles
Los Angeles, California
Chapter 120, Inflammatory back pain
Chapter 135, Clinical features of systemic lupus erythematosus

Pamela F. Weiss, MD, MSCE
Associate Professor of Pediatrics
Perelman School of Medicine at the University of Pennsylvania
Attending Physician
Division of Rheumatology
Children's Hospital of Philadelphia
Philadelphia, Pennsylvania
Chapter 110, The juvenile-onset spondyloarthropathies

Claire Y.J. Wenham, BMBS, MRCP, MD
Section of Musculoskeletal Disease
Leeds Institute of Molecular Medicine
University of Leeds
Chapel Allerton Hospital
Leeds, United Kingdom
Chapter 185, Imaging of osteoarthritis

Sterling G. West, MD, MACP
Professor of Medicine
Division of Rheumatology
University of Colorado School of Medicine
Aurora, Colorado
Chapter 175, Sarcoidosis

Harriet Branford White, MB ChB, DPhil, FRCS (Tr &Orth)
Trauma and Orthopaedic Registrar
North West Thames Rotation
St Mary's Hospital
Imperial NHS Trust
London, United Kingdom
Chapter 217, Bone tumors

Kenneth E. White, PhD
Professor of Medical and Molecular Genetics
Indiana University School of Medicine
Indianapolis, Indiana
Chapter 3, Bone structure and function

David J. Wilkinson, PhD, MRes
Research Associate
Musculoskeletal Research Group
Institute of Cellular Medicine
Newcastle University
Newcastle upon Tyne, United Kingdom
Chapter 17, Tissue destruction and repair

David E. Williams, BEng
Researcher
School of Engineering
Cardiff University
Cardiff, Wales
Chapter 6, Biomechanics of peripheral joints

Richard Williams, BSc, MSc, PhD
Associate Professor of Rheumatology
Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences
University of Oxford
Oxford, United Kingdom
Chapter 98, Animal models of rheumatoid arthritis

Hannah Wilson, BMBCh, MA Oxon, MRCS
Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Science
University of Oxford
Oxford, United Kingdom
Chapter 86, The knee

Kevin L. Winthrop, MD, MPH
Associate Professor of Infectious Diseases
Department of Public Health and Preventive Medicine
Oregon Health & Science University
Portland, Oregon
Chapter 78, Infections and biologic therapy in rheumatoid arthritis

Claudia M. Witt, MD, MBA
Professor and Chair
Institute for Complementary and Integrative Medicine
University Hospital Zurich and University Zurich
Zurich, Switzerland;
Professor of Medicine
Center for Integrative Medicine
University of Maryland School of Medicine
Baltimore, Maryland
Chapter 58, Complementary and alternative medicine

Gerhard Witzmann, MD
Senior Physician
Second Department of Medicine
Hietzing Hospital
Vienna, Austria
Appendix: Classification and Diagnostic Criteria

John B. Wong, MD
Professor of Medicine
Tufts University School of Medicine
Chief, Division of Clinical Decision Making
Tufts Medical Center
Boston, Massachusetts
Chapter 27, Principles of health economics

Anthony D. Woolf, BSc, MBBS, FRCP
Honorary Professor of Rheumatology
University of Exeter Medical School
Exeter, United Kingdom
Bone and Joint Research Group
The Knowledge Spa
Royal Cornwall Hospital
Truro, United Kingdom
Chapter 32, History and physical examination

Paul B. Wordsworth, MA, MB, BS, FRCP
Professor of Clinical Rheumatology
Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Sciences
University of Oxford
Oxford, United Kingdom;
Honorary Consultant Rheumatologist
Department of Rheumatology
Nuffield Orthopaedic Centre
Headington, United Kingdom
Chapter 215, Heritable connective tissue disorders

Jane Worthington, PhD
Professor of Chronic Disease Genetics
Institute of Inflammation and Repair
The University of Manchester
Manchester, United Kingdom
Chapter 28, Principles of genetic epidemiology

Katherine D. Wysham, MD
Clinical Fellow in Rheumatology
Department of Medicine
University of California San Francisco
San Francisco, California
Chapter 121, Clinical features of axial spondyloarthritis

Huji Xu, MD, PhD
Professor and Chairman
Department of Rheumatology and Immunology
Shanghai Changzheng Hospital
Second Military Medical University
Shanghai, China
Chapter 123, Genetics of axial spondyloarthritis

David A. Young, PhD
Senior Lecturer
Musculoskeletal Research Group
Newcastle University
Newcastle upon Tyne, United Kingdom
Chapter 17, Tissue destruction and repair

Stephen P. Young, BSc(Hons), PhD
Reader in Experimental Rheumatology
Institute of Inflammation and Ageing
University of Birmingham
Birmingham, United Kingdom
Chapter 22, Metabolomics

Md Yuzaiful Md Yusof, MBChB, MRCP
NIHR Doctoral Research Fellow
Leeds Institute of Rheumatic and Musculoskeletal Medicine
University of Leeds
NIHR Leeds Musculoskeletal Biomedical Research Centre
Leeds Teaching Hospitals NHS Trust
Leeds, United Kingdom
Chapter 75, Inhibitors of B cells

Haoyang Zhuang, PhD
Division of Rheumatology and Clinical Immunology
University of Florida College of Medicine
Gainesville, Florida
Chapter 139, Autoantibodies in systemic lupus erythematosus

Angela Zink, PhD
Head of Epidemiology Unit
German Rheumatism Research Center
Professor of Musculoskeletal Epidemiology
Charité University Medicine Berlin
Berlin, Germany
Chapter 31, European biologics registers

Preface

Rheumatology, Seventh Edition, builds on the success of the previous editions. The most notable change is the addition of Professor Ellen Gravallese as one of the Editors. Professor Gravallese is internationally recognized for her basic and translational work in the study of the immune-mediated inflammatory mechanisms of bone and cartilage destruction in rheumatoid arthritis. Her addition to the Editorial team has refocused our efforts to provide a strong basic and translational science component to the new edition.

Designed to meet the needs of the practicing clinician, this medical reference book provides extensive coverage of rheumatic and musculoskeletal diseases from basic scientific principles to practical points of clinical management in a lucid and logical manner. As stated by Professor Jan Dequeker in his review of the fourth edition, "*Rheumatology* is the most comprehensive, authoritative rheumatology text designed to meet the complete needs of all practicing and academic rheumatologists as well as arthritis-related health care professionals and scientists interested in disorders of the musculoskeletal system. The edition is firmly grounded on modern medical science, integrating the relevant basic biology with current clinical practice, easily accessible, user-friendly, and a beautifully illustrated color publication." Dr. Harry Brown, in his review of the sixth edition, noted that "[m]y lasting impression of this book was the very same as the first time I opened these two lavish volumes and that was – wow. I would suspect this is the nearest you would get to an encyclopaedia of rheumatology."

For this new edition, every chapter has been either substantially revised or, in many cases, entirely rewritten, following a rigorous editorial policy to ensure that the content and format of the book remain consistent and meet the highest possible standards. Each chapter has been updated to incorporate a broad range of new information. Seventeen completely new chapters cover basic biomedical and translational science, disease and outcome assessment, including new imaging modalities and early emerging disease, clinical therapeutics, and patient management, including rehabilitation. The text has been streamlined, ensuring that each chapter contains the most critical and current information in the field, while supplemental materials (including extra tables, figures, and bonus text) are conveniently located online. The index has also been improved, making it easier for the reader to find topics of interest.

The production of this edition of *Rheumatology* has been a greatly enjoyable team effort. We would like to thank the authors who have contributed to this and previous editions of the book, as well as the excellent team at Elsevier, including Jennifer Shreiner, Michael Houston, Nancy Duffy, Ted Rodgers, Bridget Hoette, and Nichole Beard.

We look forward to bringing you the eighth edition in another 4 years.

Marc C. Hochberg
Ellen M. Gravallese
Alan J. Silman
Josef S. Smolen
Michael E. Weinblatt
Michael H. Weisman

Acknowledgments

We would like to acknowledge the tremendous work of the contributors to this edition of *Rheumatology,* without whom this book would not have been possible. In addition, we would like to recognize our mentors: Drs. Eva Alberman, Ronald J. Anderson, and Laurie Glimcher, and the late Harry Currey, Georg Geyer, Lawrence E. Shulman, Carl Steffen, Alfred D. Steinberg, Mary Betty Stevens, and the late Nathan J. Zvaifler.

We would also like to thank our in-office editorial support (Aida Medina, Robin Nichols, Jacqui Oliver, Marion Skobek) for all of their hard work and diligence. Last, but certainly not least, we want to acknowledge our patients, who continue to provide stimulating challenges to us in our clinical practices.

Marc C. Hochberg
Ellen M. Gravallese
Alan J. Silman
Josef S. Smolen
Michael E. Weinblatt
Michael H. Weisman

Section 1

SCIENTIFIC BASIS OF RHEUMATIC DISEASE

A. Anatomy and Physiology

The synovium

Andrew Filer • Christopher D. Buckley

1

Key Points

- The synovium is a mesenchymal membrane that lines diarthrodial joints, tendon sheaths, and bursae.
- Specialized functions of synovium include nonadherence, control of synovial fluid production and composition, and providing chondrocyte nutrition.
- The normal synovium produces very low levels of proinflammatory cytokines and some antiinflammatory, proresolving cytokines and eicosanoids. In addition, the low levels of expression of RANKL (receptor activator of nuclear factor-κB ligand) with high levels of expression of OPG (osteoprotegerin) result in a low RANKL-to-OPG ratio. This homeostatic balance is likely to be important in preventing osteoclastogenesis in the normal, noninflamed synovium.
- Mesenchymal markers expressed by synovial fibroblasts, such as cadherin-11, endosialin (CD248), and podoplanin (gp38), may be critical for the development of the synovial lining by facilitating cellular organization, compaction, and matrix development. In pathologic settings, they appear to promote cartilage invasion and bone destruction.
- Synovial fibroblasts carry positional and topographic memory that may provide the molecular basis for site-specific differences in the pattern of joint involvement in different rheumatologic diseases.

DEFINITIONS

The study of synovial tissue is of major importance in understanding the pathogenesis of inflammatory arthritis, including rheumatoid arthritis (RA) and seronegative spondyloarthritis (SpA). Despite this, our knowledge of the immunohistochemical architecture of the synovial membrane, particularly in normal subjects, is surprisingly limited, mainly because of the lack of good tissue and cell-specific markers and the difficulty in obtaining synovial tissue in the early as opposed to later stages of disease.

Synovium is the soft tissue lining the spaces of diarthrodial joints, tendon sheaths, and bursae. The term includes both the continuous surface layer of cells (intima) and the underlying tissue (subintima). Whereas the intima is composed of specialized tissue resident macrophages and fibroblasts, the subintima contains blood and lymphatic vessels, a cellular content of both resident fibroblasts and infiltrating cells in a collagenous extracellular matrix (ECM). Between the intimal surfaces is a small amount of fluid, usually rich in hyaluronan (hyaluronic acid). Together, this structure provides a nonadherent surface between tissue elements. Unlike serosal surfaces, which also have nonadherent properties, synovium is derived from ectoderm and does not contain a basal lamina.

In normal subjects, the intimal layer is 20 to 40 μm thick in cross-section, and the areolar subintima can be up to 5 mm in thickness. At many sites, there is no discrete membrane, especially where subintima consists of fat pad or fibrous tissue.

Synovium is often atypical. Intimal cells may be absent. Superficial bursae contain little or no hyaluronan-rich fluid.[1] Ganglia are herniated sacs containing hyaluronan-rich fluid but do not occur at sites of mechanical shearing and do not have a typical intima and so may not be considered really to be synovial tissue. Diseased synovial tissue may lose any recognizable lining structure and may only be definable by its relation to the joint. These variations probably reflect the interplay of several factors in synovial embryogenesis and histogenesis.

EMBRYOLOGY

In the early embryonic limb bud, a central core or blastema that will ultimately form the skeleton appears. Within this core, foci of cartilage appear, each destined to become bone. Blastemal cells around cartilage foci form a perichondrial envelope showing strong CD44 expression. The area where this envelope lies between cartilage elements is known as the *interzone,* from which the synovium forms. The perichondrium, forming a sleeve around each cartilage element, subsequently invades the cartilage to form bone marrow. Thus, synovial and bone marrow stromal cells come from the same embryonic stock and this is reflected in their transcriptional functional abilities.[2,3]

Shortly before the joint cavity forms, CD55 expression appears on cells along the joint line[4] followed later by vascular cell adhesion molecule 1 (VCAM-1) expression. After cavity formation, the intimal layer also takes on a higher level of expression of CD44 and β_1 integrins compared with subintima. Expression of these three markers (CD44, CD55, VCAM-1 [CD106]) confirms the strong similarity between synovial and bone marrow stromal cells.

The mechanism of cavity formation is not fully understood; a working hypothesis implicates interactions between interzone cells bearing CD44 (a hyaluronan receptor) and hyaluronan itself.[5,6] Shortly before cavity formation, the cells of the potential joint line show high uridine diphosphoglucose dehydrogenase (UDPGD) activity, which suggests increased hyaluronan synthesis. At the time of cavity formation, high levels of hyaluronan appear along the joint line, saturate CD44, and induce disaggregation; at low concentrations hyaluronan cross-links CD44 molecules on adjacent cells, inducing cell aggregation.

Cavity formation might be expected to require lysis of matrix fibers. However, in human joints, cavity formation is not associated with high local levels of matrix metalloproteinases (MMPs) at the joint line. In fact, matrix fibers appear to run only parallel to the joint line before cavity formation; apoptotic cells found in the interzone at this time are not localized to the joint line and are unlikely to contribute to cavity formation. It appears, therefore, that development of the joint cavity arises more from differential tissue expansion than through loss of solid elements.

STRUCTURE

The microscopic anatomy of synovial tissue was first fully described by Key,[7] who divided synovium into three main types on the basis of subintimal structure: fibrous, areolar, and adipose (Fig. 1.1, *a* to *c*). He also noted that subintima may be periosteum, perimysium, or even hyaline or fibrocartilage.

Areolar synovium is the most specialized form (see Fig. 1.1, *a*). It is often crimped into folds, which may disappear when stretched. Less often it carries projections or villi. A more or less continuous layer of cells lies two or three

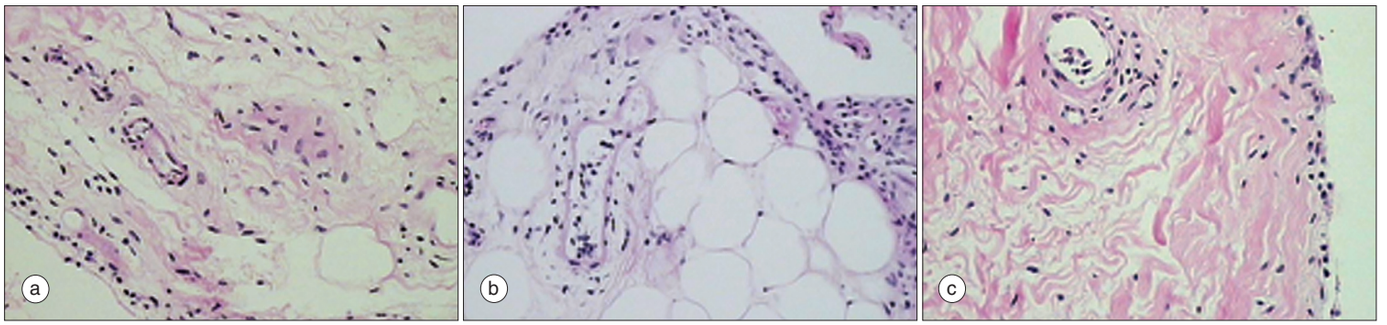

FIG. 1.1 **(a)** Areolar form of synovium (hematoxylin and eosin [H&E]). **(b)** Adipose form of synovium (H&E). **(c)** Fibrous form of synovium (H&E). (Magnification ×200.)

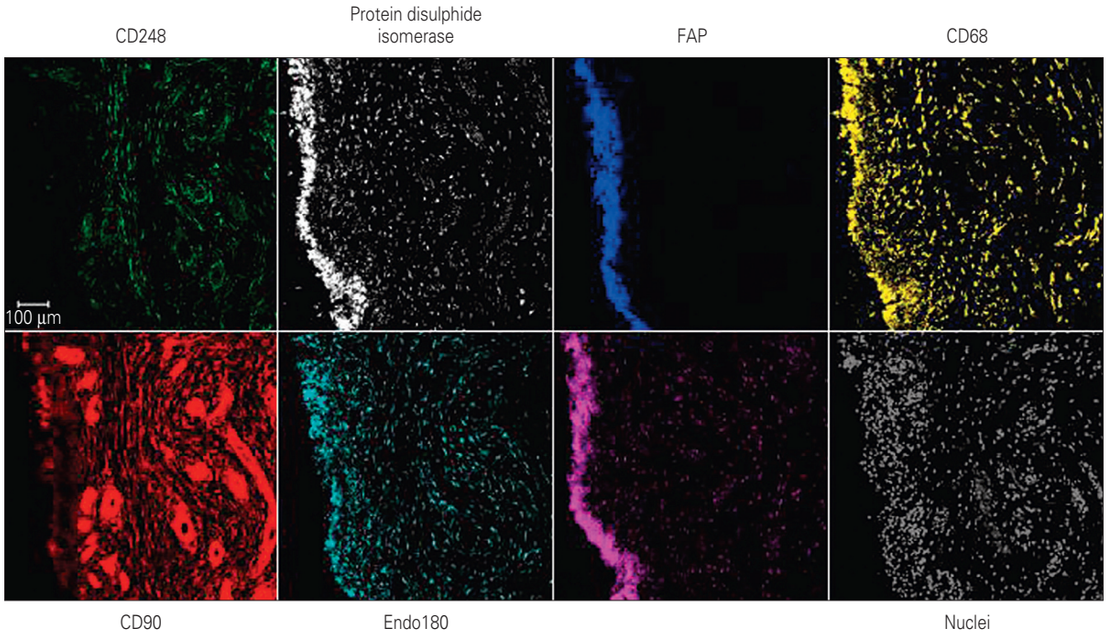

FIG. 1.2 Stromal markers differentially expressed in the lining and sublining. *FAP,* Fibroblast activation protein-α.

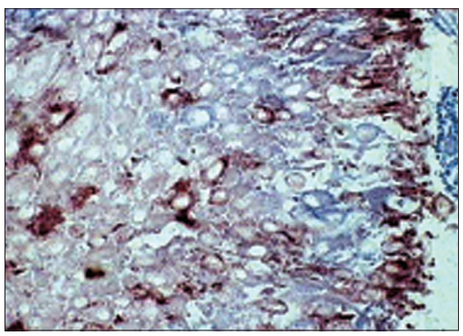

FIG. 1.3 Synovium in rheumatoid arthritis (×400) showing a thickened intimal layer containing mainly CD68+ macrophages *(red)* on the surface and weakly CD55+ fibroblastic cells beneath *(blue).*

deep on the tissue surface.[8,9] Immediately beneath these cells are capillaries. Further into the tissue, there is a plexus of small arterioles and venules,[10,11] often associated with mast cells. Lymphatic vessels can be found in all types of normal synovial tissue, although they are infrequent in the fibrous type of normal synovium.[12] In normal synovium, most lymphatic vessels are found in the deep subintima and fibrous layers, but in synovium from patients with inflammatory arthritis, lymphatic vessels are widespread and numerous. Nerve fibers are present, chiefly in association with blood vessels.[13] Three different layers of tissue matrix may be distinguished. The intima is associated with a fine fibrillar matrix with few type I collagen fibers.[14] Beneath this is a layer relatively rich in type I collagen, which forms a physical membrane. Deeper is a loose layer that allows the membrane to move freely. Beyond the loose layer lies ligament, tendon, or periosteum.

Adipose synovium occurs as fat pads and within villi (see Fig. 1.1, *b*). It has a complete intimal cell layer and a superficial net of capillaries. The intima may lie directly on adipocytes, but there is usually a band of collagen-rich substratum, but the deeper tissue is fat. Villi usually have a central arteriole and venule but can be avascular. The amount of fat in villi varies and probably decreases with age, with an increase in fibrous tissue.

Fibrous synovium is more difficult to define, consisting of fibrous tissue such as ligament or tendon on which lies an intermittent layer of cells (see Fig. 1.1, *c*). Fibrous synovium may be indistinguishable from fibrocartilage, especially in the annular pads found in finger and toe joints.

INTIMAL CELLS

Two types of intimal cells have been defined by electron microscopy, one consistent with a macrophage (type A) and the other with a fibroblast (type B).[15] It is now generally accepted—from immunohistochemical studies and other lines of evidence—that intimal macrophages are true macrophages. However, whether they are derived from circulating precursors from the bone marrow or are derived from fetal macrophages remains unclear.[16] Intimal fibroblasts on the other hand are nonhematopoietic cells and are tissue derived.[14,15,17,18] In normal healthy synovium, synovial fibroblasts are the dominant cell population.[19]

Immunohistochemical and cytochemical methods have superseded electron microscopy as tools for cell identification.[20] Intimal macrophages can be distinguished by their nonspecific esterase (NSE) activity and expression of surface markers such as CD68 and CD163. Often they are CD45 positive as well as expressing podoplanin/gp38 (Fig. 1.2). Intimal fibroblasts show intense activity of the enzyme UDPGD and prominent expression of VCAM-1 and CD55 (complement decay-accelerating factor [DAF]). In most disease states such as RA, intimal cells increase in size and number (Fig. 1.3). This is not just due to *hyperplasia* but a complex change in cell populations, in terms of both origin and function, which may be dominated by macrophage influx.[19]

SYNOVIAL MACROPHAGES

Macrophages are present in both the intima and subintima. Intimal macrophages carry typical macrophage lineage markers. They show prominent NSE activity and are strongly positive for CD163 and CD68 but less so for CD14 (Fig. 1.4). Macrophages also express the immunoglobulin receptor FcγRIIIa. Strong FcγRIIIa expression is restricted to a subset of macrophages that correspond closely to sites of macrophage activation in rheumatoid disease: synovial, alveolar, serosal, scleral, and salivary gland; lymphoid tissue and bone marrow; and Kupffer cells.[21] Subintimal macrophages are FcγRIIIa dull or negative. Macrophages also express Z39Ig, a recently described inducible cell surface receptor linked to the classic complement pathway; Z39Ig expression can occur during macrophage differentiation and induce activation of the transcription factor nuclear factor-κB (NF-κB) and production of matrix-degrading MMP-9.[22]

Macrophages make up a minority of cells in normal intima (Figs 1.4 and 1.5). In disease, the proportion of macrophages increases (see Figs 1.2 and 1.3). Distribution varies, but a common pattern is a superficial layer of macrophages with an intimal phenotype; beneath this a layer of intimal fibroblasts; and further beneath and beyond the limits of the intima, a zone of NSE-weak, strongly CD14+ and FcγRI+ macrophages, associated with venules. The deep, strongly CD14+ cells may be recently recruited cells and the superficial cells tissue resident.[23]

In addition to true macrophages, there may be a small number of antigen-presenting interdigitating dendritic cells in normal synovial intima; these are more frequent in disease with greater overlap of markers, which confounds interpretation.[24,25] Cells with features of osteoclasts such as expression of tartrate-resistant acid phosphatase and the vitronectin receptor also often appear in inflamed synovium. However, fully typical osteoclasts with calcitonin receptors appear to be restricted to pigmented villonodular synovitis and giant cell tumors of tendon sheath.

SYNOVIAL FIBROBLASTS IN THE STROMA

The anatomical term *stroma* was originally derived from the Greek word describing a platform on which to lie and is used to describe the supporting substance of a tissue. Its principal role is to maintain the microenvironment required by the parenchyma, the important functional elements of each body system. The stroma includes the cells of mesenchymal origin; the nerves, the vessels, and the epithelia that reside in a tissue in steady state; and the extracellular matrices and fluids that these cells produce. Traditionally, the diversity of stromal cells, and in particular fibroblast phenotype and function and their roles beyond those of space filling and ECM homeostasis, has been underplayed in the synovium.[26,27] We now know that these cells vary phenotypically at different anatomical sites and contribute significantly to the identity of individual tissues, providing the so-called "stromal postcode."[28] Furthermore, it is known that, rather than acting as a bystander to the body's protective mechanisms and to disease processes, the fibroblast is capable of actively participating and indeed orchestrating inflammation and immunity.[29] The fibroblast communicates with resident and infiltrating cells via cytokines and cell contact dependent mechanisms, playing a central role in the pathogenesis of synovial pathology.

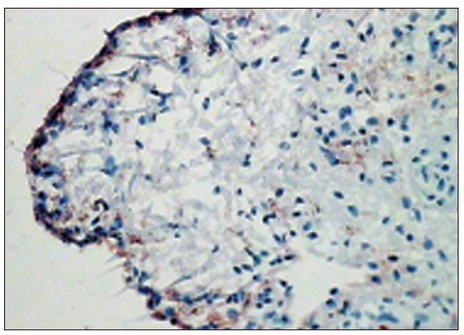

FIG. 1.4 Synovial macrophages. Normal synovium (×200) stained for CD68+ macrophages *(red)*.

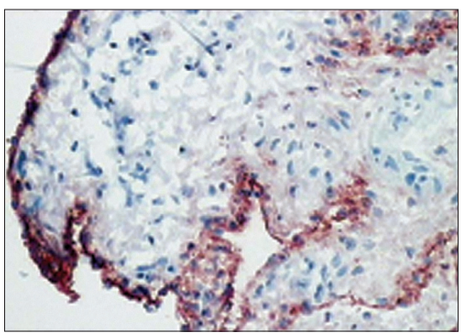

FIG. 1.5 Normal synovium (×200) stained for CD55+ fibroblasts, which are the predominant cell in the normal synovium intimal layer (contrast with Fig. 1.3).

The synovial intima contains cells that are adapted to hyaluronan production. In normal synovium, CD68-negative intimal fibroblasts express high enzymatic activity for UDPGD.[30] UDPGD converts UDP glucose into UDP glucuronate, one of the two substrates required by hyaluronan synthase for hyaluronan polymer assembly. Unlike the activity of many other enzymes, UDPGD activity in intimal fibroblasts is reduced, rather than enhanced, in diseased tissue. Synovial intimal fibroblasts express CD55 (see Fig. 1.5), a feature distinguishing them from intimal macrophages.[31,32]

Cells disaggregated from inflamed synovium and grown in tissue culture display fibroblast characteristics and ramifying processes with production of high levels of MMPs.[33] It is not known whether they derive from intimal or subintimal cells. In tissue sections, immunoreactivity for collagenase and gelatinase is patchy and not necessarily confined to the intima.

Synovial intimal fibroblasts also show prominent expression of several adhesion molecules,[9,34,35] including VCAM-1, intercellular adhesion molecule 1, CD44, and β_1 and β_3 integrins. Expression of VCAM-1 (Fig. 1.6) is particularly unusual, being absent from most other normal fibroblast populations, but CD44 and β_1 integrins are present at lower levels. The role of VCAM-1 with respect to intimal fibroblasts is puzzling, reflecting its embryologic similarities to bone marrow fibroblasts, which are also VCAM-1 positive. Expression of VCAM-1 may modulate cell trafficking because its ligand, $\alpha_4\beta_1$ integrin, is present on mononuclear leukocytes but not granulocytes. Intimal fibroblasts may allow transmigration of polymorphs but not mononuclear cells into synovial fluid, potentially trapping inflammatory cell infiltrates within the synovial membrane in disease states such as RA.

Recently, the presence of both clusterin (a glycoprotein involved in recycling and apoptosis) and (gp38) podoplanin (a membrane glycoprotein with diverse functions) has been reported in normal synovial fibroblasts; interestingly, podoplanin (which in the setting of neoplasia is associated with poor prognosis and metastatic disease) has been shown to be highly expressed in RA synovial fibroblasts with their attendant migratory and invasive potential[36,37] (Fig. 1.7).

Under inflammatory conditions, fibroblasts can act as organ-specific sentinel cells, where they play a role in the switch from acute resolving to chronic persisting. In addition to contributing to the recruitment and emigration of inflammatory cells to and out of the joint, they modulate the survival and retention of infiltrating leukocytes. Interestingly, new data raise the possibility of epigenetically programmed aggressive fibroblasts "spreading" arthritis from inflamed to uninflamed joints in the early stages of arthritis but at the same time offering the possibility of specifically targeting stromal subpopulations of choice.[38]

The expression of two other surface molecules by synovial fibroblasts is noteworthy. Complement receptor 2 (CR2, CD21) is not expressed by normal intimal fibroblasts but can be induced on synovial fibroblasts in culture, in contrast to other fibroblast populations.[39] DAF, VCAM-1, and CR2 are all involved in B-lymphocyte survival, as is a bone marrow stromal cell marker, BST-1, reported to be expressed on fibroblasts in rheumatoid, but not normal, synovial intima.[40] Other molecules associated with bone marrow stromal

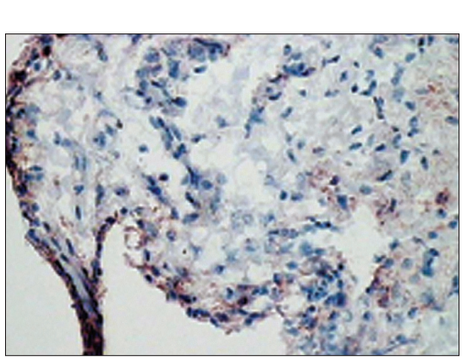

FIG. 1.6 Normal synovium (×200) stained for vascular cell adhesion molecule 1.

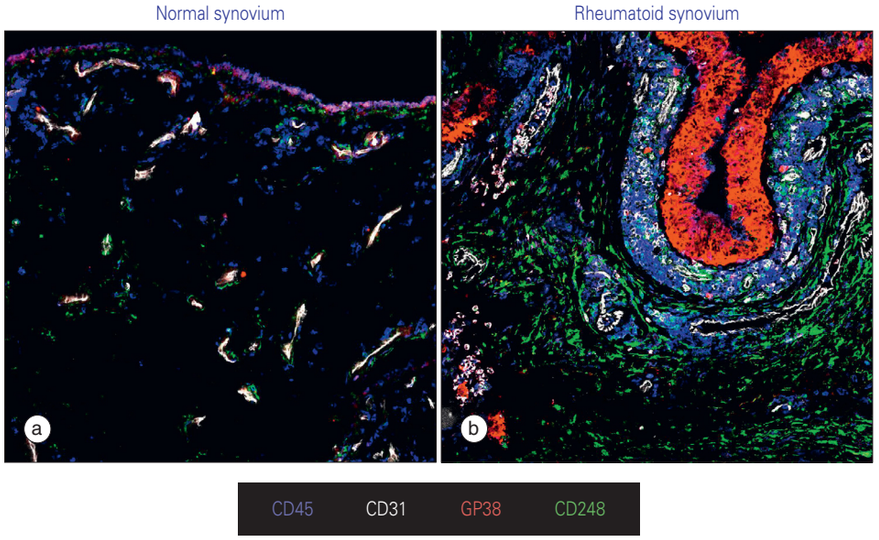

FIG. 1.7 Stromal markers differentially expressed in rheumatoid arthritis. **(a)** Normal synovium. **(b)** Rheumatoid synovium.

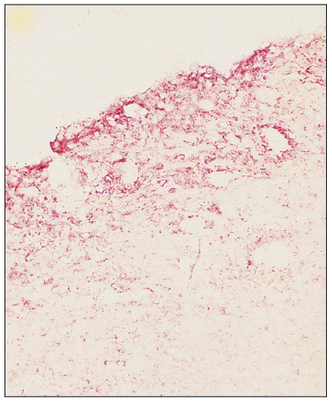

FIG. 1.8 Normal synovium stained for hyaluronan using a histochemical probe derived from proteoglycan core protein hyaluronan-binding region. Staining is most intense surrounding the lining cells and decreases further into the tissue. (Magnification ×200.)

cells such as the chemokine stromal cell–derived factor-1 (CXCL12) and bone morphogenetic proteins and their receptors,[41-43] are expressed by synovial fibroblasts under various conditions. Moreover, lubricin, otherwise known as *superficial zone protein,* a glycoprotein found in synovium and the superficial zone of articular cartilage,[44] derives from the same gene as megakaryocyte-stimulating factor. A defect of this gene leads to CACP (camptodactyly arthropathy coxa vara pericarditis) syndrome. As indicated earlier, these patterns of gene expression may reflect a common embryologic origin for synovial and bone marrow stromal cells.

Self-renewing mesenchymal stem cells that compare favorably with bone marrow–derived mesenchymal stem cells in terms of their ability to differentiate into bone, cartilage, and adipose tissue have been isolated from the normal synovium; it is unclear which component of the synovial membrane is home to these cells,[45,46] but expression of the mesenchymal stromal cell marker CD248 in the sublining layer (see Fig. 1.7) suggests that they may derive from this anatomical compartment.[47]

INTIMAL MATRIX

Intimal matrix has an amorphous or fine fibrillar ultrastructure. It is poor in type I collagen but contains minor collagens III, IV, V, and VI[48,49] as well as laminin, fibronectin, and chondroitin-6-sulfate–rich proteoglycan, which, with collagen IV, are components of basement membrane; however, the basement membrane is conspicuous by its absence beneath the intimal layer. The looser structure of intimal matrix may be explained by the absence of entactin, which links other components in basement membrane together. Intimal microfibrils are of two types: fibrillin-1 microfibrils form a basketwork around cells, and collagen VI microfibrils form a uniform mesh.

Intimal matrix contains large amounts of hyaluronan (Fig. 1.8), which tails off 20 to 50 μm deep; this possibly indicates diffusion from the surface toward clearing lymphatics.

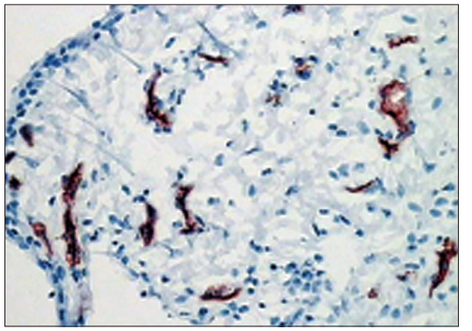

FIG. 1.9 Normal synovium (×200) stained with factor VIII to demonstrate the vascular network.

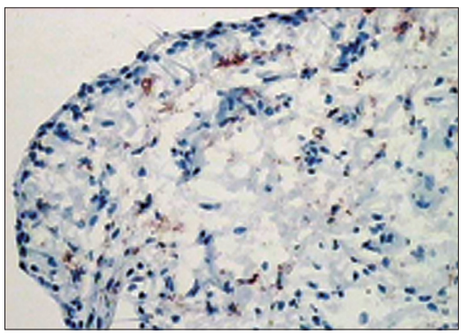

FIG. 1.10 Normal synovium (×200) stained with lymphatic vessel endothelial hyaluronan receptor-1 antibody to demonstrate the lymphatic network.

VASCULAR NET

A rich microvascular net lies beneath the synovial surface.[10,11] Capillaries (prominent in children and decreasing with age) occur just below or within the intima (Fig. 1.9). Some capillaries are fenestrated, and fenestrae tend to face the tissue surface[50]; 50 to 100 μm beneath the surface, small venules are prominent. About 200 μm beneath the surface, larger venules, together with arterioles and lymphatics (Fig. 1.10),[12] form an anastomosing quadrilateral array. Vessels with lymphatic staining characteristics are prominent in RA synovium. It has been proposed that failure of lymphatic drainage of synovial fluid is a cause of villous proliferation in RA synovial tissue. If this is correct, it is likely to be due to overloading of existing lymphatic channels with hyaluronan-rich extracellular fluid and leukocytes rather than a lack of lymphatic channels.[12]

Apart from the fenestration of superficial capillary endothelial cells, there is little evidence of specialization in synovial endothelium. Endothelial cells enlarge in inflamed tissue, and microvascular proliferation can occur, but

these events are common to inflammation at many sites. Tissue-specific adhesion molecules, or *addressins*, have been sought in the synovium, but nothing conclusive has been found unlike the case in the skin and gut, both epithelial organs.

CELL ORIGINS AND RECRUITMENT

Evidence to date indicates that both intimal and subintimal macrophages derive from bone marrow via circulating monocytes, many of which probably arrive through subintimal venules and migrate to the intima. Whether tissue resident macrophages also contribute remains unclear, but recent studies suggest that nonclassical monocytes or macrophages can contribute to the persistence or resolution of arthritis.[23]

Intimal fibroblasts are thought to arise by division within synovium. They might be a discrete self-replicating population, distinct from subintimal fibroblasts, but several pieces of evidence argue against this. Rates of cell division within the intima are very low, even in disease. After arthroplasty or synovectomy, intimal cells—likely replaced from the subintima rather than arising from intimal rests—reappear and express CD55, UDPGD, and VCAM-1. Disaggregated and cultured synovial fibroblasts lose VCAM-1 and CD55 expression, but the majority, apparently including cells of subintimal origin, readily express these markers after cytokine stimulation, in contrast to fibroblasts of dermal or subcutaneous origin. These findings suggest that synovial fibroblasts, in both the intima and subintima, belong to a specialized population with a propensity to express VCAM-1 and CD55 and are more similar to bone marrow than skin fibroblasts.[2,3]

Two studies[8,9] have demonstrated the range of cells that can be found in the synovial subintima. CD3+ T cells, including CD4+, CD8+, and memory T cells, can be found within the normal synovial tissue; although they are likely to be simply trafficking through the normal synovium, their role, if any, in the homeostasis of synovial tissue is unknown. It is also possible to detect B cells, plasma cells, and granzyme B+ cells in normal synovium, although they are present in small numbers.

Although production of inflammatory cytokines, including interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α),[9] can be detected in normal synovial tissue, expression levels are far lower than those seen in inflamed synovial tissue such as in RA. The amount of antiinflammatory cytokine production, at least in the case of IL-1 receptor antagonist (the naturally occurring inhibitor of IL-1), IL10, and other proresolving factors is far greater than the amount of inflammatory cytokine seen (Fig. 1.11).[51] This would achieve the desired result of suppressing an inflammatory process in the normal synovial tissue. Similarly, the amount of RANKL (receptor activator of NF-κB ligand, an essential factor for the development of osteoclasts) seen in normal synovial tissue is low.[9] The net result of this is to suppress the formation of osteoclasts within the normal synovium and preserve homeostasis within the normal joint.

FUNCTION

The functions of synovial tissue have proven to be remarkably difficult to define.[52] Similar to other soft connective tissue, synovium provides a deformable packing that allows movement of adjacent, relatively nondeformable tissues. The difference between synovium and other soft connective tissue is that it allows most of the movement to occur between rather than within tissues. Areolar synovium may also have specialized viscoelastic properties for coping with the stretching, rolling, and folding it undergoes during joint movement.

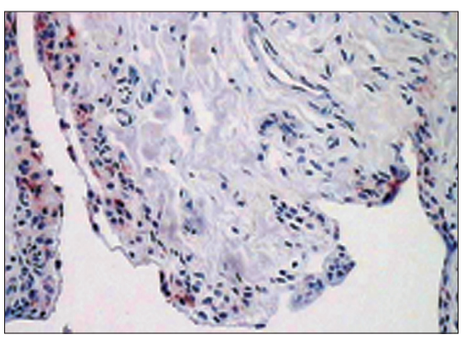

FIG. 1.11 Normal synovium (×200) stained with an antibody to detect the interleukin-1 receptor antagonist.

Functions of the tissue relating to the synovial cavity may be considered to be the following:

- Maintenance of an intact nonadherent tissue surface
- Lubrication of cartilage
- Control of synovial fluid volume and composition
- Nutrition of chondrocytes within joints

MAINTENANCE OF THE TISSUE SURFACE

Synovial surfaces must be nonadherent to allow continued articular movement. Animal models suggest that production of hyaluronan by intimal fibroblasts may be important in inhibiting adhesion.[53] Plasminogen activator and DAF from intimal fibroblasts may also inhibit fibrin formation and scarring. To retain synovial fluid, the intimal matrix consists of a fibrous mat of a particular porosity that allows free exchange of crystalloids and proteins but inhibits rapid transit of the viscous hyaluronan solution that is an important component of the fluid. The vasculature is likely to be important in both intimal cell nutrition and recruitment of new cells. New macrophages are derived from blood monocytes that are thought to enter the tissue through venules, and perivascular fibroblasts may provide the main pool of intimal fibroblast precursors.

LUBRICATION

The ability of synovial fluid to lubricate cartilage surfaces depends on the presence of glycoproteins, especially a glycoprotein known as both *lubricin* and *superficial zone protein* because of its localization to the surface of synovium and cartilage.[44]

Whatever the precise forces acting on fluid volume, the presence of hyaluronan is likely to be the main factor responsible for retaining a constant volume of fluid during exercise.[54] This fluid is probably important as a cushion for synovial tissue and as a reservoir of lubricant for cartilage. It is likely that mechanical stimulation of intimal fibroblasts dictates the rate of synthesis and exportation of hyaluronan into the synovial fluid compartment. Thus, when synovial fluid volume is high, reduced mechanical stresses on intimal fibroblasts result in a reduced rate of hyaluronan production and vice versa.

Two distinct mechanisms create joint effusions. When synovium is mechanically irritated by worn bone and cartilage, the composition of the fluid remains reasonably normal. Excessive production of hyaluronan by intimal fibroblasts stimulated by frictional forces retains plasma dialysate in the synovial cavity; in synovitis, the effusion is an accumulation of exudate similar to a pleural effusion (i.e., an overspill from the inflammatory edema in synovial tissue created by increased vascular permeability). Recent theories about possible low-grade inflammatory and immune reactions contributing to the pathogenesis of osteoarthritis suggest that these two mechanisms of effusion development may not be as distinct as originally thought.[55] Additionally, recent proteomic evidence suggests that the increased vascular permeability as seen in inflammation may be related not only to an increase in interendothelial gaps but also to glycocalyceal damage and aquaporin upregulation.[56]

CHONDROCYTE HEALTH AND NUTRITION

The synovium provides the major structure that aids chondrocyte nutrition. In normal joints, a surprisingly large proportion of hyaline cartilage lies within 50 μm of a synovial surface. In any one position, only a small proportion of cartilage is opposed to the other articular surface, and synovium packs most of the space between less congruent areas. In immature joints, the incomplete subchondral plate may contribute to nutrition, but in adult joints, this route is unlikely to be significant. Nutrition of areas of cartilage that do not come into close contact with synovium (concave articular surfaces in particular) must take an indirect route. Although a small proportion of nutrition may be imparted by smearing of a thin film of fluid over these surfaces during movement, indirect routes through cartilage matrix and the apposed articular cartilage may be more important.[6]

Diarthrodial joints (in both cartilage and synovium) have been found to express high levels of transforming growth factor-β (TGF-β), a latent complex that requires activation to induce a biologic response. Recent in vivo experiments suggest that shearing of synovial fluid because of physiologic joint motion may play an important role in TGF-β activation, which may be essential to maintain the biochemical content and structural integrity of healthy cartilage.[57,58]

Even though the blood vessels in synovium provide the most direct route for cartilage nutrition, there is no evidence that they are structurally adapted to this function. The fenestrae seen in superficial capillaries are present in

tendon sheath synovium at sites where there is no cartilage (or tendon) dependent on their supply of small molecules.

SYNOVIUM AS A TARGET FOR IMMUNE-MEDIATED DISEASE

Synovial joints are involved in several immunologic and inflammatory disorders, including RA, systemic lupus erythematosus, and seronegative SpA. Perhaps the most important reason for studying the biology of the synovium is to obtain insight into which immunopathologic processes are likely to be suitable therapeutic targets in inflammatory arthritides and in particular whether the synovial fibroblast is a therapeutic target.[2,3]

Autoimmune responses to synovial antigens might theoretically occur, but despite considerable effort, evidence for a specific synovial antigen is lacking. Moreover, associated targeting of other tissues such as pericardium or uveal tract requires an explanation. Few, if any, synovium-specific antigens are known, and when rheumatic disorders are associated with autoantibodies, the antigens involved are ubiquitous.

Understanding the microarchitecture of the normal synovium, including the wide range in microscopic appearances; cellular infiltrates; and production of cytokines, enzymes, and other biologically relevant proteins, assists in understanding the relevant changes in synovial tissue architecture and immunopathology in disease states. Although the architecture of the normal synovium is not as homogeneous as previously portrayed, consistencies across the broad spectrum of normal synovial tissues can be contrasted with those seen in chronically inflamed synovial tissue. The marked increase in synovial lining layer thickness with a reversal of the normal ratio of type A to type B intimal cells, which favors type B cells in normal synovium and type A cells in RA, is an example of this. Numerous other examples can be given, including the changes in subintimal cell content and cytokine and chemokine production,[59] vascular and lymphatic changes, and production of MMPs and stimulators of osteoclast formation.

A recent study in a mouse model of arthritis has also raised the possibility of cadherin-11 expression on synovial fibroblasts as a potential therapeutic target in the treatment of RA[60,61]; inhibition of cadherin-11 interactions in this model interfered with both synovial inflammation and cartilage invasion by pannus without having any effect on bone erosion (predominantly osteoclast dependent) or immunosuppression. In this model, inflammation could be reduced substantially by antibodies to cadherin-11 or a cadherin-Fc fusion protein. Furthermore, cadherin-11 expression has been found to promote invasive behavior of fibroblasts and is increased by IL-17 and TNF-α, cytokines very relevant in RA pathophysiology.[62-64]

SUMMARY

Despite the biologic importance of understanding how the synovium responds to damage and drives inflammation, remarkably little is known about how stromal cells (as opposed to leukocytes) change during synovial development and inflammation. Difficulties in accessing synovial tissue from normal subjects and patients with early disease and the lack of good cell markers have proved to be obstacles to such work. However, synovial stromal cells are a functionally heterogeneous group with some displaying proinflammatory and destructive properties but others being immune regulatory and helping facilitate tissue repair. This has led to a dilemma: Which stromal cells should be targeted, and which should be retained? A clear understanding of the biology and significance of synovial tissue biology is therefore essential to provide a coherent rationale for targeting stromal cells in the future treatment of patients with arthritis.

ACKNOWLEDGEMENT

The authors would like to thank Drs. Malcolm Smith and Mihir Wechalekar, whose chapter in the previous edition informed much of the present discussion.

REFERENCES

1. Canoso JJ, Stack MT, Brandt KD. Hyaluronic acid content of deep and subcutaneous bursae of man. *Ann Rheum Dis*. 1983;42:171-175.
2. Filer A, Antczak P, Parsonage GN, et al. Stromal transcriptional profiles reveal hierarchies of anatomical site, serum response and disease and identify disease specific pathways. *PLoS ONE*. 2015;10(3):e0120917.
3. Filer A, Parsonage G, Smith E, et al. Differential survival of leukocyte subsets mediated by synovial, bone marrow, and skin fibroblasts: site-specific versus activation-dependent survival of T cells and neutrophils. *Arthritis Rheum*. 2006;54(7):2096-2108.
4. Edwards JC, Wilkinson LS. Distribution in human tissues of the synovial lining-associated epitope recognised by monoclonal antibody 67. *J Anat*. 1996;188:119-127.
5. Craig FM, Bayliss MT, Bentley G, et al. A role for hyaluronan in joint development. *J Anat*. 1990;171:17-23.
6. Edwards JC, Wilkinson LS, Jones HM, et al. The formation of human synovial joint cavities: a possible role for hyaluronan and CD44 in altered interzone cohesion. *J Anat*. 1994;185:355-367.
7. Key JA. The synovial membrane of joints and bursae. In: Cowdry EV, ed. *Special cytology*. New York: PB Hoeber; 1932.
8. Singh JA, Arayssi T, Duray P, et al. Immunohistochemistry of normal human knee synovium: a quantitative study. *Ann Rheum Dis*. 2004;63:785-790.
9. Smith MD, Barg E, Weedon H, et al. Microarchitecture and protective mechanisms in synovial tissue from clinically and arthroscopically normal knee joints. *Ann Rheum Dis*. 2003;62:303-307.
10. Davies DV. The structure and functions of the synovial membrane. *Br Med J*. 1950;1:92-95.
11. Wilkinson LS, Edwards JC. Microvascular distribution in normal human synovium. *J Anat*. 1989;167:129-136.
12. Xu H, Edwards J, Banerji S, et al. Distribution of lymphatic vessels in normal and arthritic human synovial tissues. *Ann Rheum Dis*. 2003;62:1227-1229.
13. Mapp PI. Innervation of the synovium. *Ann Rheum Dis*. 1995;54:398-403.
14. Ghadially FN. Fine structure of joints. In: Sokoloff L, ed. *The joints and synovial fluid*. New York: Academic Press; 1978:105-176.
15. Barland P, Novikoff AB, Hamerman D. Electron microscopy of the human synovial membrane. *J Cell Biol*. 1962;14:207-220.
16. Perdiguero EG, Geissmann F. The development and maintenance of resident macrophages. *Nat Immunol*. 2016;17(1):2-8.
17. Edwards JC. The nature and origins of synovium: experimental approaches to the study of synoviocyte differentiation. *J Anat*. 1994;184:493-501.
18. Edwards JC, Willoughby DA. Demonstration of bone marrow derived cells in synovial lining by means of giant intracellular granules as genetic markers. *Ann Rheum Dis*. 1982;41:177-182.
19. Henderson B, Revell PA, Edwards JC. Synovial lining cell hyperplasia in rheumatoid arthritis: dogma and fact. *Ann Rheum Dis*. 1988;47:348-349.
20. Edwards JCW, Wilkinson LS. Immunohistochemistry of synovium. In: Henderson B, Edwards JCW, Pettifer ER, eds. *Mechanisms and models in rheumatoid arthritis*. New York: Academic Press; 1995:133-150.
21. Bhatia A, Blades S, Cambridge G, et al. Differential distribution of Fc gamma RIIIa in normal human tissues and co-localization with DAF and fibrillin-1: implications for immunological microenvironments. *Immunology*. 1998;94:56-63.
22. Lee MY, Kim WJ, Kang YJ, et al. Z39Ig is expressed on macrophages and may mediate inflammatory reactions in arthritis and atherosclerosis. *J Leukoc Biol*. 2006;80:922-928.
23. Misharin AV, Cuda CM, Saber R, et al. Nonclassical Ly6C(-) monocytes drive the development of inflammatory arthritis in mice. *Cell Rep*. 2014;9(2):591-604.
24. Poulter LW, Janossy G. The involvement of dendritic cells in chronic inflammatory disease. *Scand J Immunol*. 1985;21:401-407.
25. Wilkinson LS, Worrall JG, Sinclair HD, et al. Immunohistological reassessment of accessory cell populations in normal and diseased human synovium. *Br J Rheumatol*. 1990;29:259-263.
26. Patel R, Filer A, Barone F, et al. Stroma: fertile soil for inflammation. *Best Pract Res Clin Rheumatol*. 2014;28(4):565-576.
27. Naylor AJ, Filer A, Buckley CD. The role of stromal cells in the persistence of chronic inflammation. *Clin Exp Immunol*. 2013;171(1):30-35.
28. Buckley CD, Barone F, Nayar S, et al. Stromal cells in chronic inflammation and tertiary lymphoid organ formation. *Annu Rev Immunol*. 2015;33:715-745.
29. McInnes IB, Buckley CD, Isaacs JD. Cytokines in rheumatoid arthritis—shaping the immunological landscape. *Nat Rev Rheumatol*. 2016;12(1):63-68.
30. Wilkinson LS, Pitsillides AA, Worrall JG, et al. Light microscopic characterization of the fibroblast-like synovial intimal cell (synoviocyte). *Arthritis Rheum*. 1992;35:1179-1184.
31. Medof ME, Walter EI, Rutgers JL, et al. Identification of the complement decay-accelerating factor (DAF) on epithelium and glandular cells and in body fluids. *J Exp Med*. 1987;165:848-864.
32. Stevens CR, Mapp PI, Revell PA. A monoclonal antibody (Mab 67) marks type B synoviocytes. *Rheumatol Int*. 1990;10:103-106.
33. Krane SM, Goldring SR, Dayer JM. Interactions among lymphocytes, monocytes and other synovial cells in the rheumatoid synovium. *Lymphokines*. 1982;7:75-87.
34. Edwards JC. Synovial intimal fibroblasts. *Ann Rheum Dis*. 1995;54:395-397.
35. Connolly M, Veale DJ, Fearon U. Acute serum amyloid A regulates cytoskeletal rearrangement, cell matrix interactions and promotes cell migration in rheumatoid arthritis. *Ann Rheum Dis*. 2011;70:1296-1303.
36. Boland JM, Folpe AL, Hornick JL, et al. Clusterin is expressed in normal synoviocytes and in tenosynovial giant cell tumors of localized and diffuse types: diagnostic and histogenetic implications. *Am J Surg Pathol*. 2009;33:1225-1229.
37. Ekwall AK, Eisler T, Anderberg C, et al. The tumour-associated glycoprotein podoplanin is expressed in fibroblast-like synoviocytes of the hyperplastic synovial lining layer in rheumatoid arthritis. *Arthritis Res Ther*. 2011;13:R40.
38. Lefèvre S, Knedla A, Tennie C, et al. Synovial fibroblasts spread rheumatoid arthritis to unaffected joints. *Nat Med*. 2009;15(12):1414-1420.

39. Leigh RD, Cambridge G, Edwards JCW. Expression of B-cell survival cofactors on synovial fibroblasts. *Br J Rheumatol*. 1996;1:110.
40. Lee BO, Ishihara K, Denno K, et al. Elevated levels of the soluble form of bone marrow stromal cell antigen 1 in the sera of patients with severe rheumatoid arthritis. *Arthritis Rheum*. 1996;39:629-637.
41. Fowler MJ Jr, Neff MS, Borghaei RC, et al. Induction of bone morphogenetic protein-2 by interleukin-1 in human fibroblasts. *Biochem Biophys Res Commun*. 1998;248:450-453.
42. Marinova-Mutafchieva L, Taylor P, Funa K, et al. Mesenchymal cells expressing bone morphogenetic protein receptors are present in the rheumatoid arthritis joint. *Arthritis Rheum*. 2000;43:2046-2055.
43. Seki T, Selby J, Haupl T, et al. Use of differential subtraction method to identify genes that characterize the phenotype of cultured rheumatoid arthritis synoviocytes. *Arthritis Rheum*. 1998;41:1356-1364.
44. Jay GD, Britt DE, Cha CJ. Lubricin is a product of megakaryocyte stimulating factor gene expression by human synovial fibroblasts. *J Rheumatol*. 2000;27:594-600.
45. Arufe MC, De la Fuente A, Fuentes I, et al. Chondrogenic potential of subpopulations of cells expressing mesenchymal stem cell markers derived from human synovial membranes. *J Cell Biochem*. 2010;111:834-845.
46. Sakaguchi Y, Sekiya I, Yagishita K, et al. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. *Arthritis Rheum*. 2005;52:2521-2529.
47. Naylor AJ, Azzam E, Smith S, et al. The mesenchymal stem cell marker CD248 (endosialin) is a negative regulator of bone formation in mice. *Arthritis Rheum*. 2012;64(10):3334-3343.
48. Ashhurst DE, Bland YS, Levick JR. An immunohistochemical study of the collagens of rabbit synovial interstitium. *J Rheumatol*. 1991;18:1669-1672.
49. Revell PA, al-Saffar N, Fish S, et al. Extracellular matrix of the synovial intimal cell layer. *Ann Rheum Dis*. 1995;54:404-407.
50. Suter ER, Majno G. Ultrastructure of the joint capsule in the rat: presence of two kinds of capillaries. *Nature*. 1964;202:920-921.
51. Haworth O, Buckley CD. Pathways involved in the resolution of inflammatory joint disease. *Semin Immunol*. 2015;27(3):194-199.
52. Edwards JCW. Functions of synovial lining. In: Henderson B, Edwards JCW, eds. *The synovial lining in health and disease*. London: Chapman & Hall; 1987:41-74.
53. Yagi M, Mitsui Y, Gotoh M, et al. Role of the hyaluronan-producing tenosynovium in preventing adhesion formation during healing of flexor tendon injuries. *Hand Surg*. 2012;17:13-17.
54. Levick JR, McDonald JN. Fluid movement across synovium in healthy joints: role of synovial fluid macromolecules. *Ann Rheum Dis*. 1995;54:417-423.
55. Pelletier JP, Martel-Pelletier J, Abramson SB. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. *Arthritis Rheum*. 2001;44:1237-1247.
56. Shahrara S, Volin MV, Connors MA, et al. Differential expression of the angiogenic Tie receptor family in arthritic and normal synovial tissue. *Arthritis Res*. 2002;4:201-208.
57. Albro MB, Cigan AD, Nims RJ, et al. Shearing of synovial fluid activates latent TGF-beta. *Osteoarthritis Cartilage*. 2012;20:1374-1382.
58. Fava R, Olsen N, Keski-Oja J, et al. Active and latent forms of transforming growth factor beta activity in synovial effusions. *J Exp Med*. 1989;169:291-296.
59. Parsonage G, Falciani F, Burman A, et al. Global gene expression profiles in fibroblasts from synovial, skin and lymphoid tissue reveals distinct cytokine and chemokine expression patterns. *Thromb Haemost*. 2003;90(4):688-697.
60. Lee DM, Kiener HP, Agarwal SK, et al. Cadherin-11 in synovial lining formation and pathology in arthritis. *Science*. 2007;315:1006-1010.
61. Firestein GS. Every joint has a silver lining. *Science*. 2007;315:952-953.
62. Kiener HP, Niederreiter B, Lee DM, et al. Cadherin 11 promotes invasive behavior of fibroblast-like synoviocytes. *Arthritis Rheum*. 2009;60:1305-1310.
63. Park YE, Woo YJ, Park SH, et al. IL-17 increases cadherin-11 expression in a model of autoimmune experimental arthritis and in rheumatoid arthritis. *Immunol Lett*. 2011;140:97-103.
64. Vandooren B, Cantaert T, ter Borg M, et al. Tumor necrosis factor alpha drives cadherin 11 expression in rheumatoid inflammation. *Arthritis Rheum*. 2008;58:3051-3062.

2 The articular cartilage

Dick Heinegård • Pilar Lorenzo • Patrik Önnerfjord • Tore Saxne • Linda J. Sandell

Key Points

- The cells in cartilage maintain function of the extracellular matrix (ECM) via controlled turnover in response to minor damage caused by fatigue and altered load by removing malfunctioning matrix constituents by breakdown and producing new ones to achieve repair.
- The cell obtains feedback on the quality of the ECM via a number of cell-surface receptors such as integrins, DDR-2 (discoidin domain receptor 2), hyaluronan receptors, and proteoglycans with specificity for different matrix molecules.
- The composition of cartilage ECM is different close to cells in the territorial matrix than in the more distant interterritorial matrix. Composition also varies between different types of cartilage and from surface to deep articular cartilage.
- The ECM of cartilage contains a specific proteoglycan—aggrecan—that provides a very high fixed charge density and therefore an osmotic environment, with water retention being essential for tissue resilience. Aggrecan is also part of a network in which globular domains interact with other molecules.
- Fibrillar networks with collagen as the major constituent provide the tensile properties essential for load distribution and dissipation. The fibers contain other matrix proteins (e.g., those bound at their surface) that mediate interactions with other tissue structures, including neighboring fibers, which enhances their mechanical qualities.
- Cartilage ECM contains growth factors and proenzymes that are sequestered by binding to matrix macromolecules, and these substances can be released upon degradation of the carrier molecules.
- As a result of degradation of cartilage matrix, the fragments formed are released into surrounding fluids and can be used as indicators of the ongoing process, the so-called molecular marker technology.
- Fragments of ECM components can activate innate immune responses such as complement.

Articular cartilage has key roles in the function of joints. A major function is to take up and distribute load such that a given point of the underlying bone can handle very high strain. Another role of cartilage is to provide low-friction movement. One key feature of joint diseases is deterioration of joint function, which results from progressive damage to articular cartilage by degradation of the structural components important for properties of the tissue. Progressive joint destruction may eventually lead to total loss of the cartilage accompanied by alterations in underlying bone in the common disease of osteoarthritis (OA) (Fig. 2.1). Mechanisms triggering this tissue destruction are not known in detail, but it is clear that excessive load may induce a remodeling process that fails to restore normal cartilage. Stimulation of chondrocytes by cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) induces the cells to degrade their surrounding matrix and can, over an extended period, result in total dissolution of cartilage in vitro. The identity of the individual enzyme or enzymes responsible for specific fragmentation of a particular matrix protein is not known, but in some cases, candidates have been established. A repair response often accompanies the ongoing tissue destruction. In more serious cases, this response is not sufficient, and tissue failure ensues.

A prerequisite for understanding the mechanisms of tissue destruction and failed repair is to know the functions of molecules in the extracellular matrix (ECM) and how they are assembled into larger networks. It is equally important to understand the mechanisms involved in their degradation. One important and basic clinical observation in joint diseases such as rheumatoid arthritis (RA) and OA is that after joint replacement, the inflammation recedes, and the symptoms that have been plaguing patients are ameliorated or disappear in the vast majority of cases. This brings up the question of whether the components released from cartilage actually stimulate the inflammatory reaction.

The main structural entities in cartilage include aggrecan and collagen. The proteoglycan aggrecan has a primary role in taking up load and resisting deformation. The collagen network provides tensile properties, and the type VI collagen network may have a role in protecting the cell and guiding matrix assembly. A set of molecules close to the cells have specific functions in binding to particular cell-surface receptors and thereby provide signaling of conditions in the matrix.

This chapter focuses on describing the individual cartilage macromolecules and, when possible, their functional properties and their implications for tissue assembly. In some instances, candidate enzymes have been implicated in having roles in the degradation of specific macromolecules and will be discussed.

OVERALL TISSUE ORGANIZATION

The part of the matrix closer to cells, the territorial matrix, has a somewhat different composition and structure than the matrix at some distance, the interterritorial matrix (Fig. 2.2) (for references, see Heinegård and colleagues[1]). Examples of components found in both compartments are collagen type II fibers and aggrecan; in contrast, collagen type VI is found particularly in the territorial matrix, and cartilage oligomeric matrix protein (COMP) and cartilage intermediate layer protein (CILP) are primarily found in the interterritorial matrix of normal cartilage. There is also a difference in the composition of cartilage from the superficial to deep layers. In the superficial layer, collagen fibers are thinner and arranged in parallel with the surface of the tissue; in the deeper layer, the fibers are thicker and arranged perpendicular to the surface, with a transition zone in between them (Fig. e2.1; also see Fig. 2.1). The superficial part of cartilage is enriched with a number of noncollagenous molecules, notably lubricin and asporin. At the same time, other molecules are much less abundant in this part of the tissue, as exemplified by aggrecan, which is particularly enriched toward the deeper parts of the tissue.[1A] Certain molecules such as CILP are found primarily in the middle portions of articular cartilage. Although a number of cartilage components have been described in recent years, our understanding of what specific requirements and functions are met by molecules with such a restricted localization in tissue is still very limited.

Different types of cartilage have markedly different compositions. Articular cartilage has major similarities, yet multivariate analyses of proteomics data have demonstrated differences between knee and hip articular cartilage.[2]

AGGRECAN

The major structure of aggrecan, illustrated in Fig. 2.3,[1] consists of approximately 100 chondroitin sulfate glycosaminoglycan chains, each built from a disaccharide unit that is repeated some 50 times but with extensive variability. Each disaccharide contains uronic acid with a negatively charged carboxyl group and an *N*-acetylgalactosamine with a sulfate in either the 4 or the 6 position. Each chain will therefore contribute around 100 negatively charged groups. The glycosaminoglycans are linked to a serine residue of the protein core of the proteoglycan via their reducing terminal end. The chains are clustered, and clustering differs between the two regions referred to as CS domain 1 and CS domain 2 (see Fig. 2.3). There is an additional glycosaminoglycan, keratan sulfate, that has a disaccharide building block of galactose and an *N*-acetylglucosamine with a sulfate in the 6 position. These chains are shorter and particularly enriched closer to the N-terminal end of the protein core, in the so-called keratan sulfate–rich region. The aggrecan molecule is composed of some 30 such chains.

The proteoglycan core protein contains globular domains flanking the three domains carrying glycosaminoglycan chains. The one most N-terminal, the hyaluronan-binding domain (G1 globe), contributes specific high-affinity binding of the aggrecan molecule to hyaluronan (see below). After a short interglobular domain there is a second globular domain (G2) that has structural similarities to the G1 globe but does not bind hyaluronan and has no known function. In the very C-terminal end of the aggrecan molecule, there is a G3 globe with a lectin homology domain; it contributes by binding to other proteins (e.g., fibulins and tenascins), which themselves can form molecular complexes involving several such molecules (see Fig. 2.2). Recently, mutations

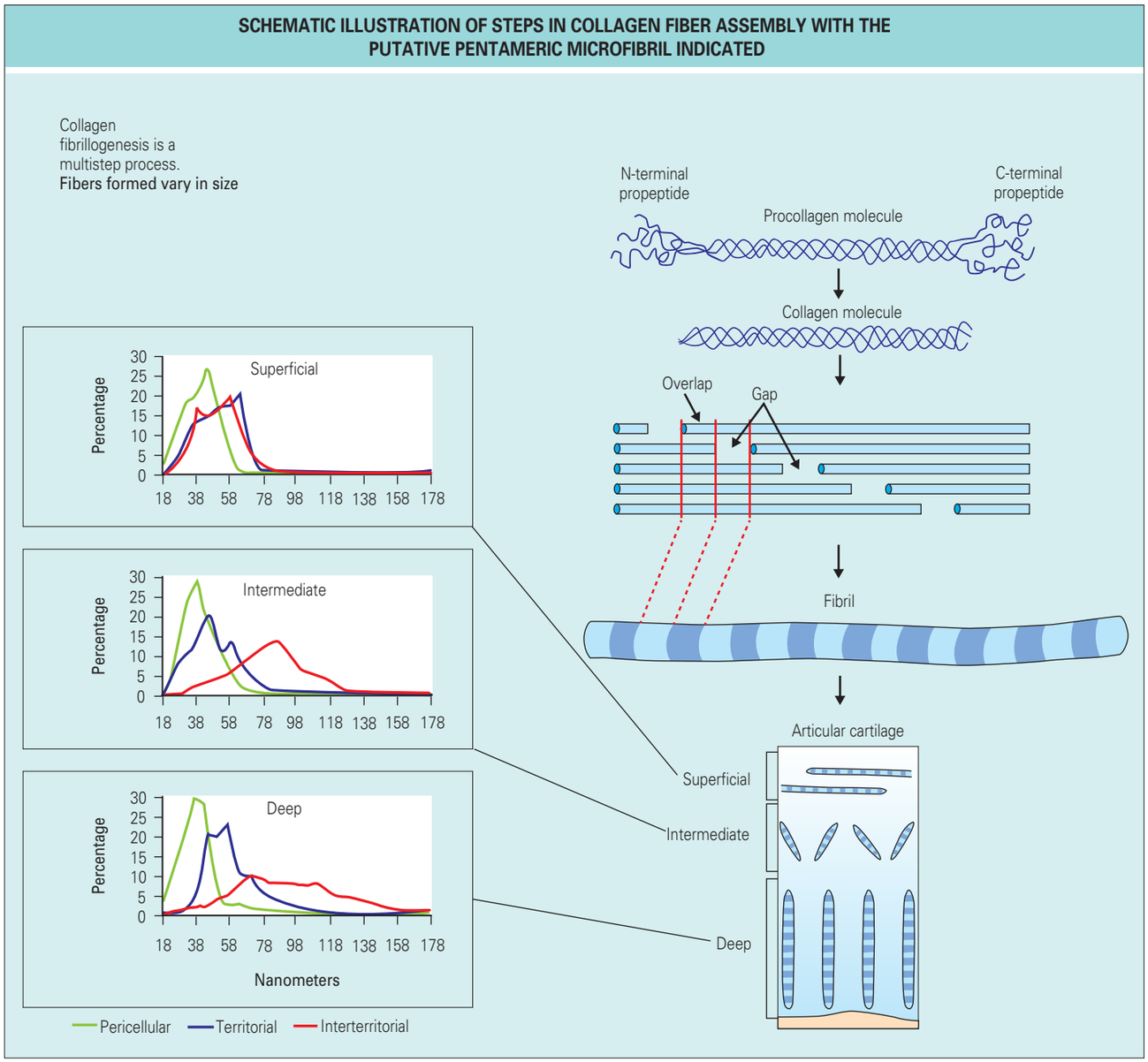

FIG. E2.1 The organization of collagen fibers at different distances from the surface of articular cartilage is shown, as well as differences in fibril diameters between the pericellular, territorial, and interterritorial compartments.

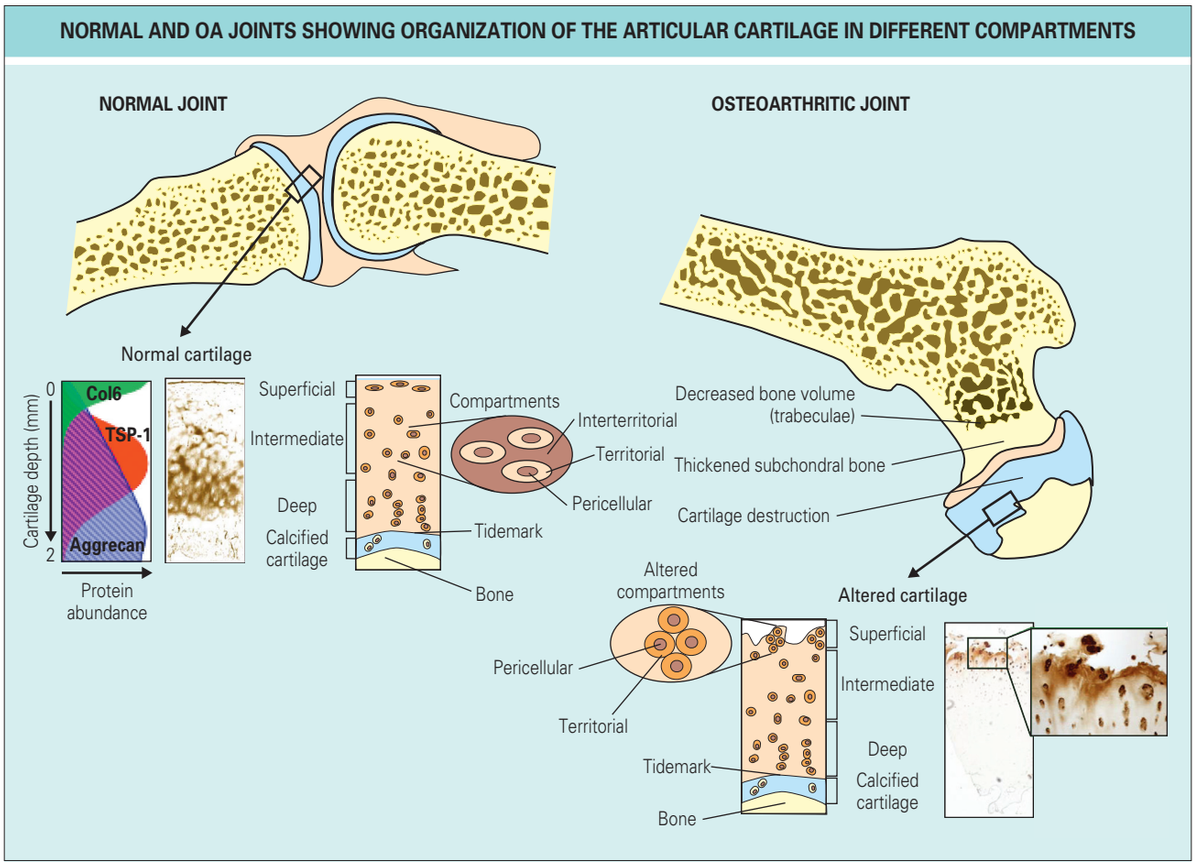

FIG. 2.1 As an example, one cartilage protein (cartilage intermediate layer protein [CILP]) shows a distinct change in localization from distribution in normal cartilage in intermediate parts of the tissue and rather selectively in the interterritorial matrix to a prominence at the superficial parts and primarily in the pericellular compartment in the diseased cartilage. The different distribution of matrix proteins with depth of the articular cartilage is illustrated by way of three examples in the *left diagram*. *OA,* osteoarthritis.

that abolish binding of the G3 domain to the fibulins and tenascins have been shown to lead to familial osteochondritis dissecans.[3]

Many aggrecan molecules will bind to a single, very long strand of hyaluronan, thereby forming large aggregates containing more than 100 glycosaminoglycan chains, each with some 100 negatively charged groups. These charges are thus fixed in the tissue, and the presence of counter ions results in an osmotic environment that retains water and has an essential role in cartilage function by resisting compression and distributing load. The interaction of aggrecan via its G1 globe with hyaluronan is stabilized by the link protein having structures similar to the hyaluronan-binding domain of aggrecan. This link protein will bind to this domain of the proteoglycan, as well as to hyaluronan.

In normal cartilage turnover, as well as in pathology, the aggrecan molecule is cleaved by enzymes called aggrecanases (i.e., ADAMTS-4 [a disintegrin and metalloproteinase with thrombospondin motifs] and ADAMTS-5)[4,5] (see Fig. 2.3). One site of this cleavage is in the interglobular domain between the hyaluronan-binding G1 globe and the G2 globe. The cleavage occurs between the amino acids EGE and ARG (for references, see elsewhere[6]). The new N- and C-terminals formed have been used to develop antibodies that recognize the fragments produced only by the cleavage. These antibodies have in turn been used to demonstrate such fragments in body fluids and tissue extracts.[6,7] The cleavage occurring in the domain carrying the chondroitin sulfate chains results in shortening of the aggrecan with ensuing decreased number of fixed charged groups (see Fig. 2.3). With aging, shorter aggrecan molecules accumulate, the extreme being hyaluronan-binding domain with no glycosaminoglycan-binding structures remaining.[8] Indeed, in adults, a major proportion of the aggrecan molecules found in the interterritorial matrix at a distance from the cells do not contain the G3, C-terminal globular lectin homology domain (Aspberg and Heinegård, unpublished observation). On the other hand, the molecules in the territorial matrix close to the cells contain this domain, probably a result of the gradual turnover of the aggrecan molecules. At the same time, there is substantial accumulation of G1 domain, apparently retained by being bound to hyaluronan.[9] This fragment can be identified in tissue either via its new C-terminal as represented by an -EGE^{373}-COOH sequence formed through cleavage by aggrecanase or via a further matrix metalloproteinase (MMP) cleavage that forms a C-terminal -PEN^{341}-COOH sequence.[10]

Even from early experiments by Thomas[11] and Fitton-Jackson and collaborators,[12] it was clear that chondrocytes have a remarkable capacity to replace aggrecan molecules removed from tissue by the use of enzymes cleaving hyaluronan. It appears that a normal chondrocyte should be able to replace even large amounts of proteoglycans lost unless suppressed by cytokines such as IL-1 and TNF-α.

There is increasing evidence that the aggrecan degradation encountered in early joint disease, such as OA, is effectively counterbalanced by increased synthesis and deposition of the molecule such that no overall loss takes place.[13,14]

COLLAGEN FIBRILLAR NETWORKS

A major function of the ropelike collagen fiber networks in cartilage is to provide tensile strength and distribute the load so that excessive local force is not applied to the underlying bone. One type of fiber contains a core of collagen type II with a minor constituent of collagen type XI connected via a number of molecules bound at its surface.

The other fibrillar network contains a core of collagen type VI that forms a filamentous network primarily in the territorial matrix. This network is connected by a set of linker molecules to other molecules in the matrix, including collagen type II fibers and the major proteoglycans. These networks are discussed separately.

FIBERS WITH COLLAGEN TYPE II AS THE MAIN CONSTITUENT

Collagen type II and collagen type XI

The major fibrillar network in cartilage contains primarily collagen type II with a minor constituent of collagen type XI.[15] A major feature of both these types of collagen is that the molecule forming the fibers is made up of three parallel, tightly associated polypeptide chains forming a very stable triple

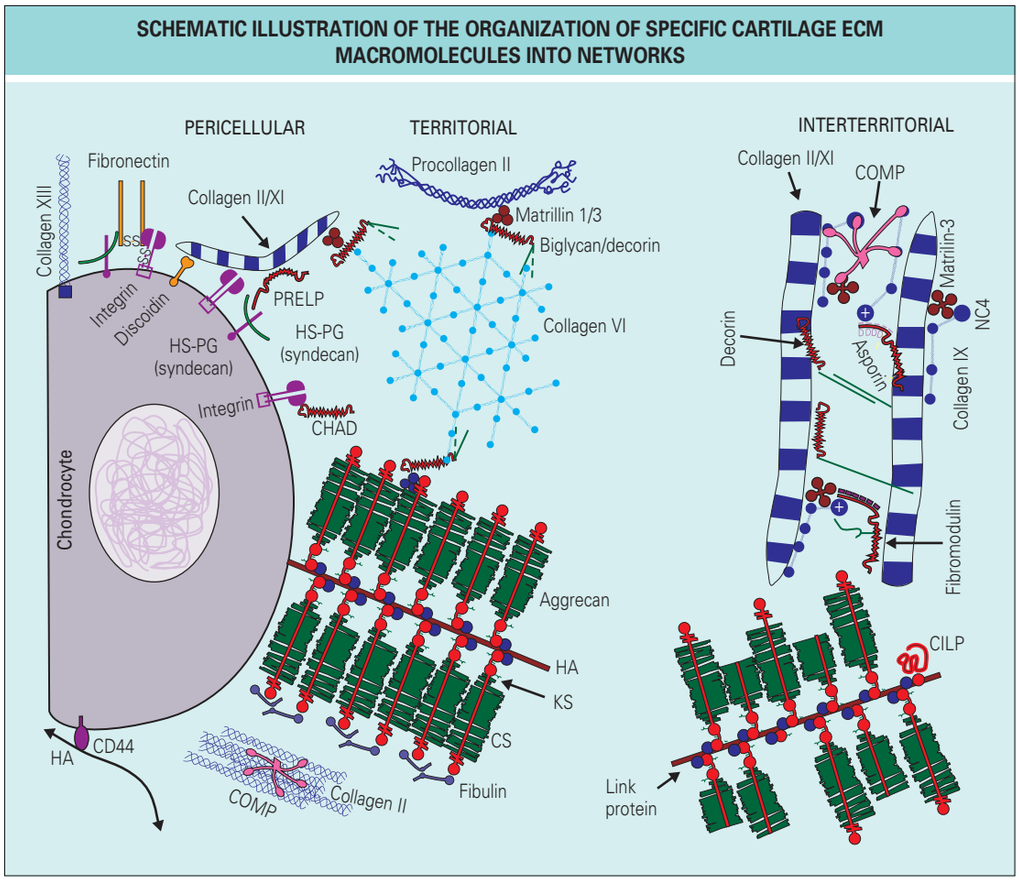

FIG. 2.2 Indicated are the interactions between extracellular matrix (ECM) proteins and specific receptors at the cell surface. Note the different molecular composition of the territorial matrix closer to the cell than in the interterritorial matrix at some distance. *CD44,* Receptor for hyaluronan; *CHAD,* chondroadherin; *CILP,* cartilage intermediate layer protein; *COMP,* cartilage oligomeric matrix protein; *CS,* chondroitin sulfate; *HA,* hyaluronan; *HS-PG,* heparan sulfate proteoglycan; *KS,* keratan sulfate; *NC4,* N-terminal globular domain of collagen 9.

helix, the collagen molecule. It is extremely asymmetric—300 nm long and 1.5 nm in diameter. An amino acid quite unique for collagen is hydroxyproline, which is essential for stability of the molecule because of the hydrogen bonds formed to the hydroxyl group. Collagen type II is produced as a procollagen that does not form fibrils until the propeptides at both ends of the C- and N-termini are cleaved off (see Fig. e2.1). After this cleavage, the molecules form fibers by interactions with other collagen molecules such that a large part of the surface of the molecule is engaged and the collagen molecules are positioned so that they form a so-called quarter stagger arrangement in relation to one another.

The assembly process is regulated by a number of molecules in the matrix, including collagen type XI. It appears that the dimensions of the fiber formed depend on the relative proportion of collagens type II and XI, with the typical ratio being on the order of 50:1. This may depend on the presence of a central core of microfibrils of collagen type II and XI that direct assembly of the fiber.[16]

Collagen fibers in the superficial and deep layers of articular cartilage are different in dimension and direction. Thus, whereas fibers in the superficial layer of articular cartilage are thin and run in parallel, the thicker fibers in the deeper parts of cartilage run perpendicular to the surface. In the transition zone layer, fibers run at an angle (see Fig. e2.1).[17] The organization can be seen as Benninghoff arcades on polarized light microscopy.

Collagen fiber formation is influenced by a number of matrix molecules, such as decorin, asporin, fibromodulin, COMP, and a special variant of an oversulfated chondroitin sulfate chain. In several of these cases, the molecules are also retained bound at the surface of the collagen fiber. This is particularly evident for collagen type IX, which has part of the molecule extending out from the fiber. These molecules appear to have roles in providing sites for interaction with other matrix molecules, including fibers other than those to which the protein is bound (see Fig. 2.2).

An important feature of the collagen fiber network is that the interactions become sealed by covalent cross-link formation after the fibers are assembled outside the cell. This cross-linking depends on the oxidation of lysine and hydroxylysine residues by lysyl oxidase to provide an aldehyde function that forms a Schiff base with a neighboring lysine amino group. These are then rearranged to become stable pyridinoline groups that cross-bridge between the molecules and within the molecules of a fiber. These cross-links are important for mechanical stability of the collagen. Because they are not metabolized on degradation of the collagen, they eventually end up in urine and can be measured as indicators of collagen breakdown.[18]

Collagen type XI

This fibril-forming collagen is an integral component of the fibers forming the main network in cartilage. Collagen type XI consists of a major triple-helical portion, similar in size to that of collagen types I and II, but in contrast to these collagens, the N-terminal propeptides are retained with the molecule incorporated into the fiber. Some reports have indicated that the retained N-terminal parts are exposed at the surface of the fibers, with collagen type II being the major constituent and the major triple-helical portion being located more centrally in the fiber.[19] Collagen type XI together with collagen type II appears to form the initial assembly of microfibrils that regulate further assembly of the cartilage collagen fiber, at least in skeletal morphogenesis.[16] Interestingly, collagen type XI forms cross-links to primarily other collagen type XI molecules.[20] There are examples of mutations in collagen type XI chains with ensuing major growth disturbances, thus indicating a role in cartilage growth and stability.[21]

Collagen type IX

This molecule is a member of the FACIT collagens (fibril-associated collagens with interrupted triple helices) and is found in tissue bound at the surface of the fibrils, with collagen type II being the major constituent. Collagen type IX contains three different α chains with three triple-helical domains (col1, col2, and col3), each surrounded by a noncollagenous domain (NC1, NC2, NC3, and NC4). The NC4 domain with its adjacent col3 triple helix protrudes from the fibers and is available for interactions with other molecules in the ECM, as schematically illustrated in Fig. 2.2.[22] Examples of such ligands are COMP and the tyrosine sulfate domain of fibromodulin. Collagen type IX often contains a chondroitin sulfate side chain bound at the NC3 domain. Its role in function of the collagen is not known.

Functionally, collagen type IX has been shown to interact with matrilins, COMP, and in particular, collagen type II. The collagen is actually covalently cross-linked to collagen type II in the fibers of adults.[23] When collagen type IX is added in vitro to fibril-forming systems of collagen type II, assembly and fiber formation are retarded.

Mutations in collagen type IX lead to severe growth disturbances, such as pseudoachondroplasia or multiple epiphyseal dysplasia. Some of these

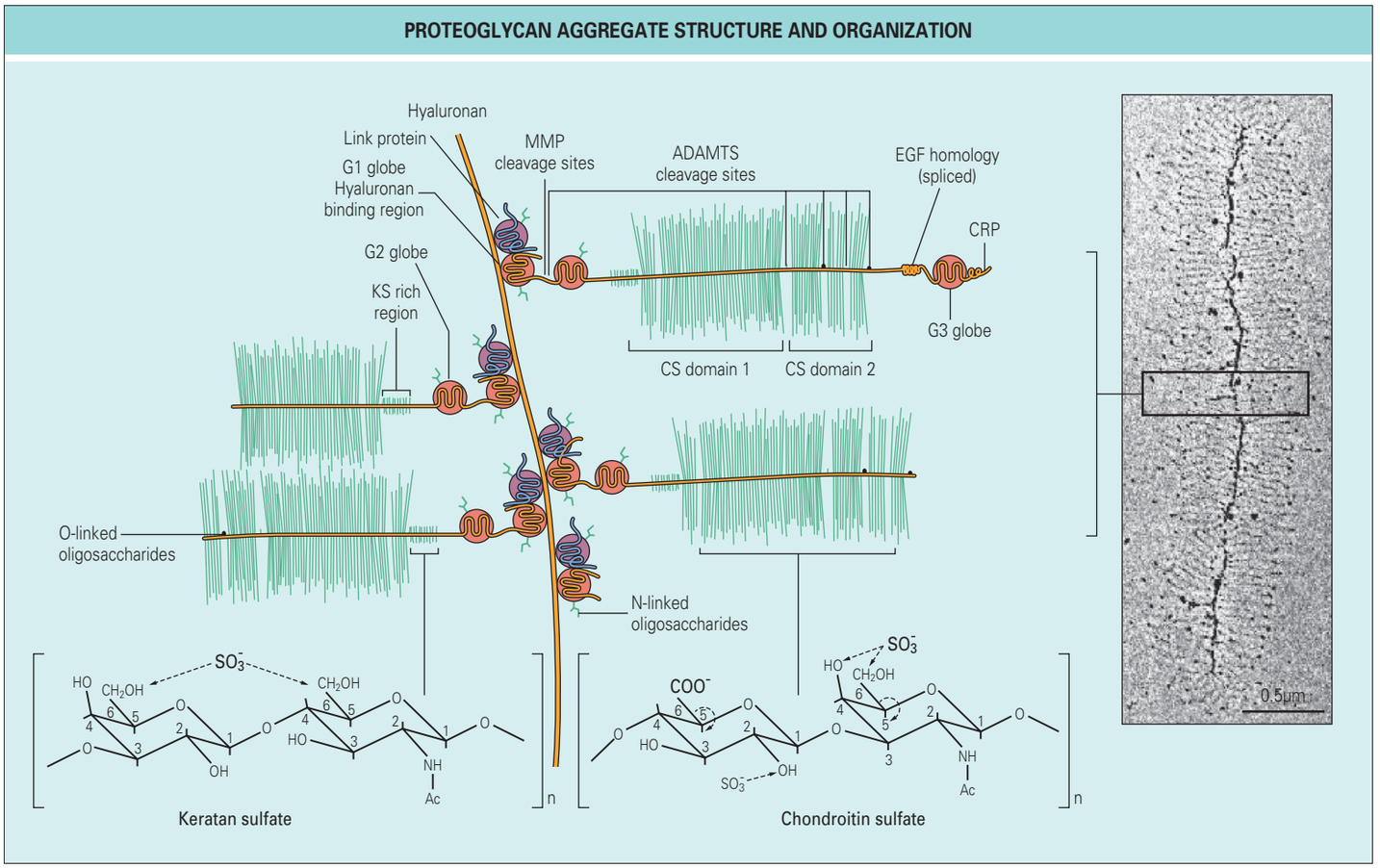

FIG. 2.3 Depiction of the structure and organization of the proteoglycan aggregate. Also shown is a rotatory shadowing electron micrograph (courtesy of Matthias Mörgelin) of an aggregate isolated from tissue. The sites for degradation by the primary active ADAMTS-4 and ADAMTS-5 are indicated, as well as one site for cleavage of matrix metalloproteinase (MMP). *CRP,* C-reactive protein; *CS,* chondroitin sulfate; *EGF,* epidermal growth factor.

disturbances are similar to those with a mutated COMP molecule, which is of special interest in view of the high-affinity interaction that this protein shows with all four NC domains of collagen type IX. Furthermore, early lesions of articular cartilage similar to those found in OA develop in mice with knockout of collagen type IX.[24]

MOLECULES REGULATING COLLAGEN FIBER ASSEMBLY

The dimensions and orientation of collagen fibers in tissue vary between different layers of articular cartilage. Moreover, fibers in the territorial matrix close to cells are thinner and have similar dimensions in different layers of cartilage. In contrast, fibers in the interterritorial matrix are thicker and have larger and more variable diameters in the deeper layers. This regulation of fiber diameter is achieved by a number of macromolecules that bind to collagen. The exact role of individual molecules in achieving the final dimensions and direction of the fibers is not clear, although there are a number of examples in which inactivation of individual genes of the involved proteins leads to altered dimensions of collagen fibrils. It is notable that asporin, similar to decorin, inhibits collagen fiber formation and that levels of these proteins are selectively very high in superficial parts of the articular cartilage, where collagen fibers are thin.[25]

The extremely long half-life of collagen type II, in excess of 100 years,[9,26] indicates that very little collagen is eliminated over the life of an individual; however, at the same time, fibers defective as a result of fatigue have to be repaired. It is possible that the very variable dimensions of the fibers in the interterritorial matrix result from adding newly synthesized collagen molecules at the fiber surface and thus gradually increasing the diameter to provide mechanical stability.

A number of molecules bound at the surface of collagen fibers are likely to prevent further accretion of collagen. It is plausible that these molecules will need to be removed before new collagen molecules can be added to a fiber for remodeling or repair.

It appears that even though the collagen itself does not turn over, molecules on the fiber's surface are continuously removed and replenished.

Molecules with putative roles in regulating fibril formation are found among those that can bind collagen in vitro.

Cartilage oligomeric matrix protein

Cartilage oligomeric matrix protein is a molecule primarily found in cartilage, where it is quite abundant at a concentration of around 0.1% of the tissue's wet weight. The molecule is made up of five identical subunits, each with a molecular weight of around 87,000 Da. The five subunits are held together by a coil-coiled domain close to the N-terminal end, and disulfide bridges further stabilize the binding. The subunits are made up of several modules, including some binding calcium. At the C-terminal end, there is a globular domain that is involved in interactions with other proteins in the matrix. The molecule can be viewed as a bouquet of tulips tied together at their stalks (see Fig. 2.2).[27]

Cartilage oligomeric matrix protein, also referred to as thrombospondin-5, is a member of this family of proteins. Cartilage also contains other thrombospondins that share the same properties, with thrombospondin-1 and thrombospondin-4 being particularly abundant. These thrombospondins, however, contain an extension beyond the coiled-coil domain in the N-terminal that has a heparin-binding motif, in this manner adding additional interacting sites. Whereas thrombospondin-4 contains five identical subunits, thrombospondin-1 contains only three.[1]

The three-dimensional structure of the C-terminal domain of thrombospondin-1 has been resolved, and its organization has been found to be stabilized by a large number of calcium ions.[28] Because the C-terminal domain of the various thrombospondins shows a great deal of conservation, it is likely that its structure is similar in all five members of the family.

Cartilage oligomeric matrix protein has been shown to bind to collagens type I and II,[1] where each individual C-terminal globular domain provides high affinity in the nanomolar range. Four binding sites are evenly distributed

along the collagen molecule. There is one at each end and two positioned along the filament such that the distance is similar between the four binding sites. Even though each COMP molecule has five identical binding sites, an individual molecule can engage only one binding site on each collagen molecule and not span the distance between two such sites. Therefore, each COMP molecule has the potential to bind to five different collagen molecules. The quarter stagger arrangement of collagen in the fiber and the fact that a pentameric microfibril unit appears to exist[29] may relate to the four similarly spaced collagen binding sites in the molecule. COMP accelerates and provides faster collagen fibril formation in vitro. It appears that this effect is mediated by the COMP molecule bringing together several collagen molecules to facilitate their interactions in the forming fiber. The COMP molecule does not remain bound directly to the surface of the forming fiber. The molecule thus appears to function as a catalyst to enhance fibril formation.

In the cartilage of growing individuals and notably in growth plates, COMP is primarily localized close to the cells in the territorial matrix, where it may have a role in stimulating collagen fibril formation.[30]

Cartilage oligomeric matrix protein has the ability to interact with all four NC domains of collagen type IX with similar high affinity in the nanomolar range. The interaction is mediated via the C-terminal globular domains (see Fig. 2.2).

In adults, COMP is localized primarily in the interterritorial matrix and may be bound to collagen type IX or one of the matrilins, which in turn bind to the surface of the collagen fiber (see Fig. 2.2). The role of COMP in adult cartilage appears to be stabilization of the collagen fiber network.

Mutations in the calcium-binding domain of COMP, as well as in the C-terminal domain, have been shown to lead to severe growth disturbances in the form of pseudoachondroplasia or multiple epiphyseal dysplasia. A feature of these conditions is that material retained in the endoplasmic reticulum of chondrocytes contains both COMP and collagen type IX.[31]

On the other hand, the COMP-null mouse shows no detectable alteration in phenotype.[32] It is possible that other molecules compensate for the lack of COMP function in such mice.

Cartilage oligomeric matrix protein is significantly upregulated in early stages of OA, even long before diagnosis, in an apparent attempt at repair. At the same time, the protein already deposited is cleaved and released from the tissue. Indeed, assay for such fragments released into body fluids has been used to measure altered cartilage metabolism as a biomarker for arthritic disease.[33]

Decorin

Decorin was the first molecule in the leucine-rich repeat (LRR) protein family to be cloned and sequenced. This family of molecules in the ECM contains four subclasses with a total of 12 members, all of which appear to share the function of binding to collagen (see Fig. e2.2).[1]

One functional domain of these molecules is a central LRR region, where residues, particularly leucine, are found at conserved locations in each repeat of some 25 amino acids, albeit somewhat variably long. Most of these molecules have 10 to 11 such repeats, and the entire domain contains a disulfide loop structure at each end. One subgroup contains molecules with only six repeats.

For further details on decorin, see ExpertConsult.com.

Decorin binds tightly to the fibril-forming collagens with a K_D in the nanomolar range. Binding is close to the C-terminus of the collagen as shown for collagen type I. Binding occurs via the LRR region and particularly involves repeats 4 and 5.[1] The critical sequence has been identified as SYIRIADTNIT.[36]

Via its binding to collagen, decorin inhibits the formation of collagen fibers in vitro in a dose-dependent manner. Accordingly, mice lacking decorin as a result of knockout technology have irregular collagen fibers with increased diameter, particularly prominent in skin.[37] They do not show increased early joint pathology, thus indicating that other molecules may compensate for the lack of decorin in articular cartilage.

Decorin is bound to collagen fibers in tissue, with the glycosaminoglycan chains being free to interact with other molecules. Thus, decorin can cross-bridge to neighboring collagen fibers, as well as to other molecules in the local environment (see Fig. 2.2).

Fibromodulin and lumican

Fibromodulin and lumican belong to the same subclass of LRR proteins but with a gene arrangement distinct from that of decorin. Other members of this subgroup are keratocan, osteoadherin, and PRELP. All these molecules except PRELP contain tyrosine sulfate residues in the N-terminal extension. Notably, the number of such sulfate residues is variable both with regard to the relative proportion of candidate tyrosine residues that are sulfated within a given molecule and with regard to the number of such tyrosine residues that may carry a sulfate. One extreme is represented by fibromodulin, which contains up to nine sulfate residues; osteoadherin contains up to eight, lumican up to four, and keratocan only one.[38] PRELP, in contrast, contains a cluster of basic residues that contribute heparin-binding activity for this molecule.[39]

Collagen binding of fibromodulin has been studied extensively. It has been shown that the molecule inhibits fibril formation in vitro. Fibromodulin-null mice show altered collagen fibril dimensions, particularly apparent in the tail tendon. Unexpectedly in view of the inhibitory effects observed in vitro, the tendon contains a much larger number of thin fibrils. An explanation appears to be a higher abundance of the related lumican molecule, which could be shown to bind to the same site on the collagen, albeit with somewhat lower affinity. It thus appears that lumican may guide early events in fibril formation. The molecule may then be competed away by fibromodulin to introduce a different function. Because the mRNA levels and therefore synthesis of lumican were lower in null mice, it appears that the higher levels of this protein were caused by retarded elimination apparently secondary to lack of competition by fibromodulin.[1]

These findings illustrate that fibril formation takes place in many steps involving a set of different molecules with different roles.

Fibromodulin is also present on collagen fiber in tissue, where it is bound in the gap region.[1] It appears to be bound via its protein core with exposure of a keratan sulfate chain, as well as the tyrosine sulfate domain, which then become available to interact with PRELP and the NC4 domain of collagen type IX located on neighboring fibers. Such interactions are important for the stability and properties of the collagen network.

Fibromodulin is a target in joint disease. In a model of articular cartilage destruction caused by stimulation with IL-1 in explant culture, we have been able to show that fibromodulin is degraded after aggrecan and that the molecule is initially cleaved by MMP-13 to release almost the entire N-terminal tyrosine sulfate domain while the remainder of the molecule is initially retained in the tissue, probably bound to the collagen.[41] This loss of the anionic domain is likely to alter the properties and interactions of fibromodulin. At the same time, MMP-13 can release the entire NC4 domain of collagen type IX from cartilage.[42] This results in loss of function, with impaired noncovalent associations between collagen fibers most likely leading to impaired maintenance of structure and function of the cartilage. This may represent a central mechanism in the swelling and surface fibrillation observed in early OA.

It is of interest to note that the particular fragment retained in the tissue is found only in pathologic and not in normal tissue, although there is continuous turnover of matrix constituents in response to altered load, including removal of damaged components. This normal turnover appears to involve different mechanisms of cleavage.

Other leucine-rich repeat proteins

PRELP is distinguished by having a basic, heparin-binding N-terminal domain.[43] This mediates binding to molecules with heparan sulfate side chains, including perlecan and cell-surface syndecan and glypican. Simultaneously, the protein binds via its LRR domain to two sites on fibril-forming collagen types I and II. Thus, the molecule has the potential to bridge from the collagen network back to the cell surface. It consequently has the potential to provide feedback to cells on the condition of the matrix. Little is known of alterations in PRELP in joint disease. An interesting observation is that the isolated heparin-binding domain of PRELP binds to osteoclast precursors via cell-surface proteoglycans. The peptide becomes internalized and translocates to the nucleus, where it inhibits nuclear factor-κB (NF-κB), the target of receptor activator of NF-κB ligand (RANKL), an important stimulator of osteoclast development. The effect of the peptide is thus to inhibit osteoclast formation. Indeed, PRELP has been used to prevent the development of osteoporosis in ovariectomized mice.[44]

Chondroadherin is a cell-binding protein that forms its own subclass. The protein is further discussed later.

Asporin is a close relative of decorin but differs in having a variably long polyaspartate sequence in the N-terminal end. The number of aspartate residues varies among individuals.[45] It has been demonstrated in studies of several cohorts of individuals in Asia that OA is overrepresented in individuals with 14 such residues versus those with 13 residues in the asporin N-terminal structure.[46] In a different study from the United Kingdom, no such pronounced relationship could be discerned, thus indicating that other factors are also involved.[47] One function of the polyaspartate sequence appears to be calcium binding, for which there may be a difference between the 13 and 14 aspartate variants.[48] At the same time, asporin binds to collagen at the same sites that decorin does,[48] which can facilitate its fixation to collagen fibers in the tissue. The molecule may thus have roles in regulating mineralization, which is relevant to the development of OA. Asporin, similar to decorin, biglycan, and fibromodulin, appears to bind transforming growth factor-β (TGF-β).

There is a set of LRR proteins with only six repeats.[1]

One variable of almost all the molecules in the family is an N-terminal extension of generally less than 20 amino acids, which may contain a variety of substituents (see Fig. e2.2). Some of the molecules also have a C-terminal extension. There is also variation in glycosylation of the repeat domain, which usually contains a few N-linked oligosaccharides. In some of the small LRR proteins, such as fibromodulin and lumican, some of the oligosaccharides may contain a variably long array of 6-*O*-sulfated lactosamine repeat disaccharides (see Fig. 2.3) extended to form the glycosaminoglycan keratan sulfate.

The three-dimensional structures of decorin[34] and biglycan[35] have been resolved by x-ray crystallography. These molecules contain one (decorin) or two (biglycan) glycosaminoglycan chains bound at the N-terminal extension. These chains are chondroitin sulfate or dermatan sulfate, depending on the tissue, though very similar between the molecules in a given tissue. In articular cartilage, the chain is a low-epimerized dermatan sulfate in which a few of the glucuronic acid residues have been epimerized to iduronic acid, thereby increasing its structural variability. This glycosaminoglycan has the capability of specific interactions with other molecules in the matrix, including binding other dermatan sulfate chains.[1]

Based on the x-ray crystallographic data presented, the LRR family of molecules forms a curved structure in which two molecules form a dimer in the crystals that overlap in opposite directions in about 50% of their length such that the N-terminus of one molecule is located in the middle of the curved domain of the other. Support for such a dimeric structure was obtained by other techniques such as gel filtration with online dynamic light scattering.[34] Electron microscopy indicates that decorin and biglycan, as well as chondroadherin, may also exist in a monomeric form at very low concentrations (Mörgelin and Heinegård, unpublished work).

The presence of the proteoglycan as a monomer or a dimer has specific relevance for interactions and functions. Thus, a monomeric molecule has only one of each interacting site, but a dimer may exhibit two of each binding site.

The function of the domain with clustered tyrosine sulfate residues is becoming clearer. The domains in fibromodulin and osteoadherin mimic heparin in many interactions and bind growth factors (e.g., fibroblast growth factor-2 [FGF-2]), cytokines (e.g., oncostatin M and IL-10), MMPs (e.g., MMP-13 for fibromodulin), and a number of matrix proteins via their heparin-binding domains (e.g., PRELP and chondroadherin).[40] Thus, the fibromodulin on a collagen fiber appears to extend its tyrosine sulfate domain, which can bind to molecules with cationic domains. One such charged structure is the NC4 domain of collagen type IX, which has been shown to interact with the anionic N-terminal extension of fibromodulin,[40] as schematically outlined in Fig. 2.2.

All the molecules in this family appear to bind to collagen via their LRR domain with dissociation constants ranging from 1 to 10 nM.[1] Only in a few cases has it been established exactly where along the collagen that the LRR protein binds.

Such proteins include epiphycan, mimecan, and opticin. They all contain N-terminal extensions carrying glycosaminoglycan chains (epiphycan), tyrosine sulfate residues (mimecan), or *O*-glycosidically linked oligosaccharides (opticin) (see Fig. e2.2). Mimecan has also been named osteoglycin. There is limited knowledge on alterations of these proteins in joint disease. They do show interesting differences between different types of cartilage. Mimecan is present in articular cartilage, menisci, and intervertebral disks but virtually absent in nasal and tracheal cartilage, but epiphycan is prominent in the latter cartilage and absent from the others. Opticin is particularly prominent in connective tissues other than those of joints.

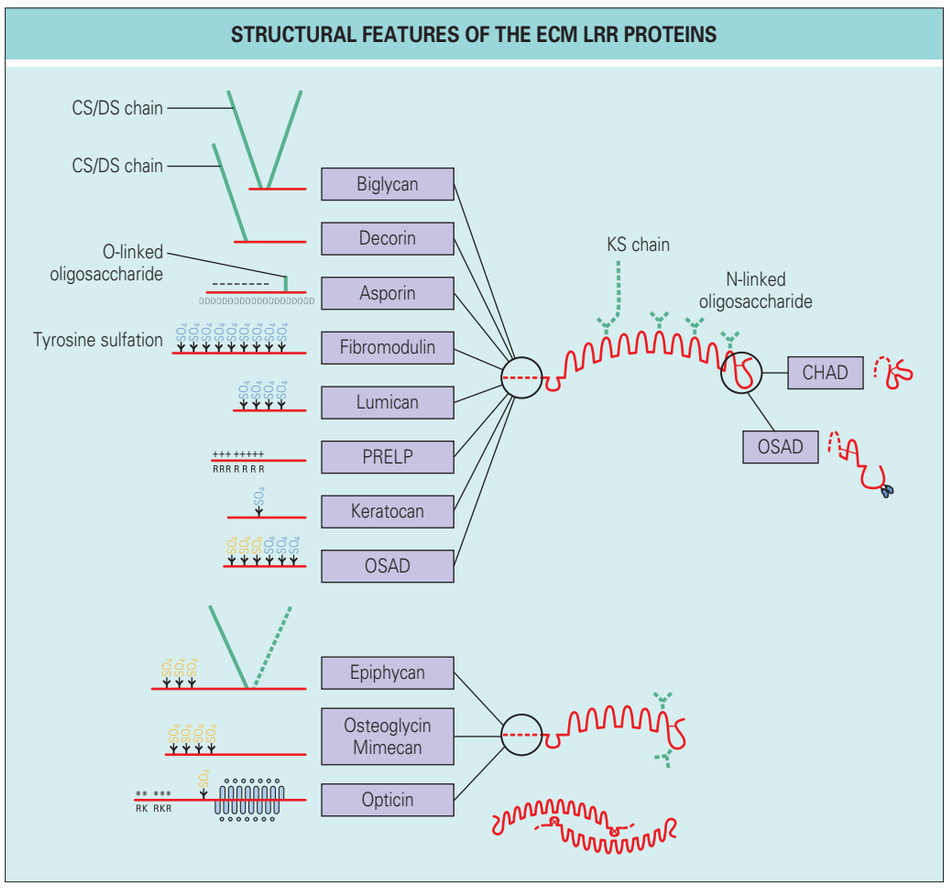

FIG. E2.2 Note the variability in the N-terminal domain between molecules, which shows negatively charged groups of different character (glycosaminoglycan, tyrosine sulfate, polyaspartate, or a cluster of basic amino acids). The C-terminal extensions of two members have distinct features. The dimeric form found for decorin and biglycan on x-ray crystallography is illustrated schematically. *CHAD,* Chondroadherin; *CS,* chondroitin sulfate; *ECM,* extracellular matrix; *LRR,* leucine-rich repeat; *OSAD,* osteoadherin.

Heparin and heparan sulfate

A glycosaminoglycan not prominent in cartilage is heparan sulfate, which has structural features overlapping those of heparin. Heparan sulfate is found as side chains of extracellular perlecan (discussed later), which has a very large protein core with several domains interacting with a number of other proteins in the ECM. The proteoglycan contains an N-terminal domain with some three glycosaminoglycan chains and a C-terminal domain with up to two chains. These chains may be heparan sulfate or chondroitin sulfate (discussed later).

Heparan sulfate contains two types of disaccharide repeats consisting of either a glucuronic acid and an *N*-acetylglucosamine or an iduronic acid and an *N*-acetylglucosamine. The hexosamine carries an *O*-sulfate group, and some of the residues have an additional *N*-sulfate instead of the *N*-acetyl group. Also, the uronic acid may be sulfated, usually in its 2 position. Thus, there is extensive variability in the building blocks, which are then assembled in variably long stretches with different repeat stretches of low- and high-sulfated residues.

Other heparan sulfate–containing proteoglycans are found at the cell surface of the chondrocyte. These include four different syndecans,[49] which contain a domain intercalated in the cell membrane, and some six glypicans, which are joined via a glycosylphosphoinositide linkage.[50] The syndecans contain an intracellular signaling domain, and as discussed later, they are involved in a number of cell reactions, and bound molecules can induce signal transduction.

FIBERS AND NETWORKS WITH COLLAGEN TYPE VI AS THE MAIN CONSTITUENT

There is a different fibrillar network in cartilage with a more restricted distribution in tissue. It has collagen type VI as a major constituent and is primarily present in the territorial matrix surrounding the cells (see Fig. 2.1).

Collagen type VI

Collagen type VI has a distinctive network of beaded filaments. This molecule contains three different α chains with a central triple-helical domain flanked by globular domains. The N-terminal portion, particularly the α_3(VI) chain, contains nine von Willebrand factor A (vWFA)-like repeats. In addition, the C-terminal portions of all three chains contain two vWFA repeats, as well as other motifs with less clear functions. The vWFA domain is found in many proteins, where it is involved in protein–protein interactions.[51]

While still within the cell, two collagen type VI molecules associate in an antiparallel fashion such that the dimer is flanked by the N-terminal domains, and the two C-terminal domains are placed so that they form two interior globular structures along the filament formed by the two triple helices. Two such dimers associate laterally to form a tetramer, with the globular domains at each end representing a pair of the vWFA-rich globules. Internally in the triple-helical central filament are found the two globular structures representing the C-terminals.[51] The tetramer is secreted from the cell, and its N-terminal structures lay the ground for further associations with fibrils involving both end-to-end and side-to-side interactions. This assembly process appears to be governed by other molecules, particularly members of the LRR protein family.

Biglycan and decorin

Biglycan and decorin both bind with high affinity to the collagen type VI N-terminal domain, independently of their glycosaminoglycan side chains. This binding is a prerequisite for formation of the collagen type VI beaded filament network in vitro. Regulation of collagen type VI assembly also depends on the presence of the chondroitin–dermatan sulfate side chains, where the two present in biglycan provide more efficient filament formation than the single chain in decorin does. The two closely related proteoglycans appear to bind to the same site, which interestingly does not seem to involve the triple-helical domain in this collagen.[52-54]

Matrilins

Cartilage matrix protein (CMP, matrilin-1) was the first of the four matrilins identified. These proteins (for references, see Klatt and colleagues[55]) contain vWFA domains. Matrilin-1 has two such domains in each of the three identical subunits with a molecular mass of around 50,000 Da; matrilin-3, with four subunits, has only one such domain.

Matrilin-1 and matrilin-3 are quite restricted to cartilage and show similar distribution between different tissues; the others have a more general distribution. Interestingly, matrilin-1 is even further restricted and not found in articular cartilage and intervertebral disks, but it is particularly prominent in tracheal cartilage. The protein is present in the more immature cartilage of the femoral head during earlier phases of development and can be seen in the early bone anlagen. Its role in cartilage is becoming clearer, and data indicate roles in collagen network function and integrin binding (for references, see Klatt and colleagues[55]). Its two vWFA homology domains (only one in matrilin-3) may mediate the ability of the protein to bind to collagen. Matrilin-1 was initially isolated because of its apparent ability to bind to aggrecan.

Mutations in matrilin-3 can induce multiple epiphyseal dysplasia or pseudoachondroplasia with skeletal malformations, similar to those caused by mutations in the interacting partners of the proteins, such as collagen IX.[56]

Matrilin-1 can be isolated from cartilage as a mixed polymer that contains subunits of matrilin-1, as well as matrilin-3. The functional significance of this heterocomplex is not understood.[57]

A module cross-linking to other matrix constituents

Decorin and biglycan appear to also have roles in the completed network. Collagen type VI isolated from chondrosarcoma cartilage–like tissue contains biglycan or decorin bound at the N-terminal globules. The proteoglycan in turn has a bound member of matrilin-1, -2, or -3. This interaction is tight with a dissociation constant on the order of nanomolar. The matrilin, in turn, binds to a procollagen type II molecule or a completed collagen fiber. Alternatively, matrilin can bind to an aggrecan molecule (see Fig. 2.2).[54] Thus, collagen type VI seems to be a center in a scaffold that binds to the other major networks in the matrix. Collagen type VI is found only in the territorial matrix closer to cells and is absent from the interterritorial matrix at some distance from the cells. Information on alterations in collagen type VI in joint disease is limited, but it is important to note that the protein is found to be particularly enriched in tissue subjected to load.

MOLECULES INTERACTING AT THE CELL SURFACE AND MODULATING CHONDROCYTE BEHAVIOR

It is important to realize that chondrocytes have the ability to both degrade the matrix and replenish lost molecules with new constituents in a process of remodeling the tissue in response to material fatigue or to altered load. The cells are guided in this endeavor by receptors at their surface that recognize specific molecules in the matrix (see Fig. 2.2). Binding elicits specific signals that will either induce cellular spreading and migration by engaging the cytoskeleton or lead to alterations in transcription and protein synthesis. Other stimuli that affect cells include mechanical forces, which provide signals that crosstalk with those from other interactions at the cell surface. Indeed, some data indicate that some of the signals elicited by mechanical load involve integrins.[58]

INTEGRIN BINDING PROTEINS

The integrins contain an α chain noncovalently bound to a β chain. The cells in connective tissues contain either a β_1 or a β_3 chain in conjunction with one of many α chains. The various integrins have different preferred ECM ligand proteins.[59] As an example of the organization of integrins, members of one such family with four members bind to collagens. They contain a β_1 chain in combination with an α_1, α_2, α_{10}, or α_{11} chain. These various integrins have different tissue distributions such that the one with α_{10} appears to be unique to cartilage, but the others have more ubiquitous distributions. Their binding to collagen may elicit different responses and result primarily in either altered production of enzymes degrading the matrix or production of building blocks such as collagen molecules.

A number of factors limit our ability to discern which integrins are present on chondrocytes in tissue. One factor is limited accessibility by the antibodies used, and another is changes in the presence of integrin during the long procedure for cell isolation. Thus, the current information on the presence of integrins at the cell surface is variable. It is likely that a number of integrins change their expression on chondrocytes in normal and in pathologic tissue in response to environmental factors.

Many of the molecules binding to integrins are not unique for cartilage. The collagen-binding integrins do not appear to be specific for a particular fibril-forming molecule. One exception is chondroadherin. This protein appears to be restricted to cartilage and can bind $\alpha_2\beta_1$ integrin but not other integrins containing the same β chain.

Chondroadherin

Chondroadherin is a member of the LRR protein family that is in its own subclass. This protein differs from other LRR family members in that the C-terminal cysteine loop is double, and the protein has a short C-terminal extension of basic amino acids. The protein has no N-terminal extension.

Chondroadherin specifically binds to cell-surface $\alpha_2\beta_1$ integrin[1,60] via a sequence in one of its C-terminal disulfide loops. Interestingly, integrin binding elicits signals that do not induce cell spreading. The very C-terminal short extension peptide of chondroadherin contains a different cell-binding sequence that engages the heparan sulfate chains of cell-surface proteoglycans, notably syndecans.[61] Binding leads to cell spreading, as well as enhanced integrin activity. The combined engagement of integrins and heparan sulfate leads to cells forming focal adhesion complexes.

Chondroadherin is present in articular cartilage but virtually absent from menisci. The protein is particularly enriched during growth in the prehypertrophic zone of the growth plate where cell multiplication is slowed, in line with its in vitro effects on cell behavior. Interestingly, the protein appears to be lost early in the process of joint damage in OA (unpublished observations).

Fibronectin

Fibronectin[62] is present in most tissues and can actually form its own fibrils, which appear to have roles in guiding matrix assembly and cell migration. The protein contains two identical subunits held together by disulfide bonds close to their C-terminal end.

Fibronectin contains a collagen-binding domain with preference for denatured collagen (gelatin). There are integrin-binding domains in which the RGD (arginine–glycine–aspartic acid) sequence represents the classic motif of integrin binding. This motif in fibronectin preferentially binds $\alpha_5\beta_1$ integrin, although $\alpha_v\beta_3$ integrin can also interact. Fibronectin also has other integrin-binding domains.[63]

Another motif is represented by the two heparin-binding domains on each subunit, each some 20 kDa. These domains can interact with heparan sulfate proteoglycans at the cell surface, including syndecan, which also represent signaling molecules.[64]

Although the details are not known, fragments of fibronectin (e.g., those containing either of the heparin-binding domains), when added to cartilage in explant culture, as well as injected into the joint, will stimulate chondrocytes to produce proteases and induce cartilage breakdown.[65,66] In contrast, the intact fibronectin molecule will not have such effects on cells. It is possible that fragmentation of fibronectin is one mechanism for propagating joint destruction in disease. The active fragments have not yet been identified in body fluids.

Fibronectin is already upregulated in articular cartilage at very early stages of OA in many species, including humans.[13] The functional consequences of this upregulation are not known but may represent part of an attempt at repair. A result is further availability of fibronectin, which may be fragmented to further increase tissue degradation.

COLLAGEN AND THE DISCOIDIN DOMAIN 2–CONTAINING RECEPTOR

Collagen has an additional cell receptor for discoidin domain receptor 2 (DDR-2). This receptor has been shown to be upregulated in mice in which OA is developing. DDR-2 will bind collagen type II, thereby inducing upregulation of MMP-13 production.[67] Exposure of collagen type II may be enhanced by removal of proteins bound at the fibrillar surface, thus making the molecule available for interactions.

HYALURONAN

Hyaluronan is an extremely long glycosaminoglycan chain that is distinct from all the others in that it is not bound to a protein core. It does not contain any sulfate groups. The backbone is made up of one to several thousand repeating disaccharides of glucuronic acid and *N*-acetylglucosamine. As discussed earlier, hyaluronan specifically binds to a domain in aggrecan that is essential for the formation of aggregates.

Hyaluronan also interacts with specific cell-surface receptors. These receptors are CD44 molecules[68] that interact with a minimally long hexasaccharide sequence of the polymer. The interactions with hyaluronan are important for organizing the pericellular environment and providing signals to the cell. CD44 occurs in many different splice forms with variable presence on different cells, but a role for such variability in joint disease in unclear. Another receptor for hyaluronan is RHAMM,[69] also a signaling receptor and, similar to CD44, it is present on a variety of cells.

LIGANDS FOR HEPARAN SULFATE/SYNDECANS

In addition to the heparin-binding domains present in fibronectin, various other matrix proteins contain such domains. Examples are most members of the thrombospondin family, with the exception of COMP, many MMPs, CILP, and PRELP.[1] As discussed earlier, isolated heparin-binding domain fragments can have variable effects on, for example, cells, depending on their fine structure. Effects range from inducing cartilage breakdown in vitro and in vivo to decreasing bone breakdown by targeting osteoclast precursors.

Interestingly, as discussed earlier, the N-terminal tyrosine sulfate domain of fibromodulin can mimic heparin in many interactions and bind other proteins to crossbridge networks, as well as sequester growth factors.

OTHER MOLECULAR FUNCTIONS

In joint disease, inflammation is a frequent component causing pain and limiting function. The inflammation is usually chronic, and one issue is whether components from cartilage can propagate the inflammatory activity. It is a long-standing observation that when cartilage is removed in joint arthroplasty, the inflammation recedes, thus indicating that factors released from the tissue have a role in propagating inflammation.

It has been shown that some patients with RA have circulating antibodies to collagen type II. Furthermore, an arthritis condition can be elicited by injecting mice and rats with collagen type II (collagen-induced arthritis). The disease can be transferred by antibodies to specific collagen type II epitopes.[70] It is hence possible that antibody binding activates complement and inflammation.

More recent findings indicate that matrix proteins may activate innate immunity. Fibromodulin has been shown to be as active as immune complexes[71] in activating the classical pathway of complement, although it does not bind to the same site. Fibromodulin binds to the head domains of C1q rather than to the triple-helical stalk region with ensuing deposition and activation of C4 and C3.

Interestingly, fibromodulin can also recruit factor H by binding to a different site than that for C1q and thereby inhibit further activation of the complement cascade. Whether different fragments released in disease will have different roles in complement activation remains to be shown.

In other experiments, it has been demonstrated that biglycan can also bind the triple-helical stalk of the complement factor C1q and in doing so inhibit activation of the classical pathway of complement. It appears that other LRR proteins such as biglycan and decorin can also bind factors involved in regulating the complement cascade. One example is a tight interaction with C4BP having a role in regulating complement activity.[72]

Cartilage oligomeric matrix protein is a potent activator of the alternative pathway via its C-terminal globular domain. Interestingly, this activity can be observed in synovial fluid from subjects with both OA and RA, as well as in blood in the form of COMP-C3b complexes created between the activator COMP and the C3b fragment formed.[73]

BINDING AND SEQUESTERING GROWTH FACTORS IN THE EXTRACELLULAR MATRIX

The ECM of cartilage contains a number of factors that are sequestered and thereby bind to specific interaction partners. On degradation of the matrix, one can predict that these factors are released and affect cellular activities. In particular, a number of growth factors have been found to have the capacity to bind to matrix proteins.

Transforming growth factor-β

Transforming growth factor-β has been shown to bind to a number of matrix proteins, and binding to some members of the LRR protein family has been investigated in particular. All three TGF-β variants bind to bacterially expressed decorin, biglycan, and fibromodulin, as well as to these proteins with the full range of posttranslational modifications. More recently, there are indications that asporin can also bind TGF-β. It has been demonstrated that active TGF-β is released from decorin on treatment with MMP-3. Indeed, cartilage contains substantial amounts of TGF-β, which has been extracted and purified from the tissue.

Fibroblast growth factor

Some matrix proteins contain heparan sulfate side chains, which are likely to bind growth factors within the FGF family. In the case of syndecan at the cell surface, these side chains appear to be involved in presenting the growth factor to its receptor.[74]

OTHER MOLECULES IN THE EXTRACELLULAR MATRIX

Perlecan was found to have a central role in cartilage development when a mouse with the gene inactivated was developed. Most of these mice die during early development as a result of problems with the heart and major

blood vessels, but those that survive until birth show major growth disturbances with an extensively altered growth plate lacking a large proportion of the collagen fibers.[75] Further studies have revealed that chondroitin sulfate side chains specific for perlecan can actually accelerate and catalyze collagen fiber formation.[76]

GP-39 is a protein upregulated in OA. It shares homology with a chitinase, but the true activity of the protein is not known.[77] GP-39 is additionally expressed in a number of other tissues, particularly in disease.

Cartilage matrix also contains a number of proteins that are made elsewhere and are normally found primarily in the circulation. There is a preference for certain proteins, and low-molecular-weight basic proteins (e.g., lysozyme) in particular appear to bind to the matrix.[78] Whether these molecules contribute specific functions is not known.

FRAGMENTS OF MATRIX PROTEINS RELEASED DURING CARTILAGE BREAKDOWN AS MOLECULAR INDICATORS OF DISEASE

In processes resulting in destruction of cartilage tissue, proteolytic enzymes degrade ECM proteins. Some of the fragments formed will no longer be retained in the tissue but are released to surrounding body fluids. By using sensitive immunoassays, such fragments can be quantified in synovial fluid or serum. This so-called molecular marker technology (biomarkers) is intended to provide new means of assaying ongoing active processes in articular cartilage. With further development, such techniques may be used in diagnosis, for estimation of the activity of the tissue-destroying process, to document effects of therapeutic intervention, and, most importantly, to discover processes during the early stage before clinical symptoms become apparent. One example is the demonstration that COMP can be used to identify patients who will have the most extensive joint destruction in both OA and RA.[33,79] Also, a number of collagen fragments created by cleavage with collagenases, as well as by subsequent gelatinase activity, have been used to monitor disease.[80,81] With further development of the technology, we hope to be able to identify indicators specific to a particular pathologic process in a given tissue. Thus, it should be possible to identify the activity of a process in the meniscus on the one hand and activity in cartilage on the other.

As discussed earlier, some of the fragments released will activate immune responses with the potential to further enhance disease activity.

REPAIR AND REGENERATION OF CARTILAGE

Cartilage ECM molecules have extremely long half-lives, and the cells of cartilage are not renewed. In addition, it is difficult to imagine how newly synthesized molecules would fully and functionally integrate into the cartilage matrix. However, anecdotally, it is believed that some individuals can repair cartilage to a limited extent, and there are animal models that demonstrate genetic differences in the ability to repair cartilage.[82] Although the genetic differences are not known for sure, some growth factors involved in development of cartilage appear to play a role, such as the Wnt family, or TGF-β.

When cartilage is damaged, a number of repair strategies have been developed, including (1) replacing bad cartilage with good cartilage from the same joint, (2) using patient chondrocytes to make new ECM, (3) attempting to recruit or implant patient stem cells, and (4) other strategies that attempt to replace the cartilage matrix with an artificial matrix. We will briefly review recent advances in these technologies.

REPLACING CARTILAGE

Intact cartilage from the same joint is used to replace damaged cartilage in surgical techniques including mosaicplasty or surgery using morcellized cartilage. The primary surgical technique is called osteochondral autologous transplantation (OAT) mosaicplasty.[83] This technique involves an open or arthroscopic transplantation of multiple cylindrical osteochondral grafts from the relatively less weight-bearing periphery of the articular surface to the cartilage defect, thus providing a hyaline cartilage–covered resurfacing.[84] OAT mosaicplasty has demonstrated acceptable long-term clinical outcomes, given the appropriate indication for surgery, with a limitation being the defect size.[85]

In another surgical technique, osteochondral allograft transplantation is also used. If the cartilage defect is too large for an autograft or a patient has failed a cartilage repair procedure, then a fresh osteochondral allograft (OCA) is a single-stage technique for large osteochondral defects, particularly in a setting of extensive subchondral bone loss.[86] Chondrocyte viability seems to be very important in this technique, and fresh OCAs stored at physiologic temperature have the highest level of chondrocyte viability. A study of 60 patients with femoral condylar allografts showed 95% graft survival rate at 5 years and 85% at 10 years.[87]

MICROFRACTURE AND AUTOLOGOUS CHONDROCYTE IMPLANTATION

This has become a substantial research area in the field of cartilage repair. Two surgical techniques are used, microfracture (MF) of the subchondral bone to recruit bone marrow mesenchymal stem cells to populate the cartilage defect and a related technique, autologous chondrocyte implantation (ACI), a two-part procedure in which chondrocytes are isolated from intact non-weight-bearing regions of the joint, expanded and dedifferentiated in the laboratory and redifferentiated and reimplanted into the osteochondral defect in the weight-bearing region.[88,89] The MF technique is considered the first-line treatment given its minimally invasive nature, technical ease, limited surgical morbidity, and relatively low costs.[90] In a study with 7-year follow-up, 80% of patients rated themselves as improved with patients younger than 35 years showing the most improvement.[91] Biopsies after MF have noted that approximately 10% had hyaline cartilage, with the majority having predominantly fibrocartilage.[92] In general, patients demonstrated clear improvement in knee function at 24 months after MF but inconclusive durability and treatment failure beyond 5 years.

Autologous matrix–induced chondrogenesis combines MF surgery with the application of a bilayer collagen membrane that physically stabilized the clot and may guide and enhance marrow-derived repair. In a study using a BST-CarGel (Piramal Like Sciences, Bio-Orthopaedic Division), treatment with MF resulted in greater lesion filling and superior repair tissue quality compared with MF treatment alone; however, clinical symptoms were equivalent between groups.[93]

Autologous chondrocyte implantation is approved for use in the United States and is most useful for younger patients who have single defects larger than 2 cm. Disadvantages include the need for two-stage procedures and an open arthrotomy, expense, and a significant rate of reoperation for graft hypertrophy, specifically with first-generation ACI treatments. Second- (porcine membrane) and third-generation (matrix-associated) ACI treatments show promise but are not approved in the United States at this time. In a series of more than 200 patients treated with ACI for larger lesions, ACI provided durable outcomes with a survivorship of 71% at 10 years and improved function in 75% of patients.[94] Magnetic resonance imaging findings confirmed complete defect filling in half of patients at final follow-up.

STEM CELLS

The limited expansion capacity of de-differentiated chondrocytes and their increasing inefficiency at redifferentiation during extended culture has led to the use of mesenchymal stem cells (MSCs) for autologous cartilage repair of larger defects.[95] MSCs are found in numerous human tissues, including bone marrow, adipose tissue, articular cartilage, and synovial membrane.[96] Cell-based therapy is becoming an established element of modern health care and is predicted to grow as knowledge and implementation of cell biology, biomaterials, and regenerative medicine increases. MSCs are defined as adherent self-renewing, fibroblastoid-like cells that can differentiate into osteoblasts, adipocytes, and chondrocytes in vitro.[97] These characteristics are present in the MSCs derived from the bone marrow, adipose tissue, and synovial joint tissues. These cells can be differentiated in vitro to chondrocytes and used as chondrocytes or can be injected into the joint, where they may differentiate into chondrocytes or, more likely, provide factors and immune inhibitors that facilitate joint repair.[98] Bone marrow MSCs were found to be at least as effective as chondrocytes for articular cartilage repair in improving symptoms of patients. However, chondrogenically induced bone marrow MSCs hold the inherent risk of either forming transient fibrocartilaginous repair tissue or undergoing terminal differentiation to form calcified cartilage, subchondral bone overgrowth, or intralesional osteophyte formation.[99] Thus alternatives to bone marrow MSCs are sought.

Adipose-derived MSCs, now called adipose-derived stem cells (ASCs), offer the advantage that they are abundant in adipose tissue or liposuction samples.[100] Two recent reviews summarize efforts to fabricate or regenerate articular cartilage using scaffolds and growth factors with ASCs.[101] Cells from the synovium, synovial fluid, periosteum, the infrapatellar fat pad, trabecular bone, and muscle have also been used to differentiate chondrocytes, but no definitive positive regeneration has been documented. There is the possibility of recruiting these native cells from the tissues to the cartilage defect, but this has not yet been productive. The area of cell-based strategies for cartilage repair is thus under intensive study. However, the field has not

moved as fast as was predicted, primarily because of safety, cost, efficacy, and regulatory hurdles.[102]

TISSUE-ENGINEERED CARTILAGE

A variety of combinations of MSCs with various scaffolds using biomaterials approaches, bioreactors, and combinations of cartilage- and bone-inducing materials have been developed. For a review, please see Boushell et al.[103]

ACKNOWLEDGEMENT

This chapter originates from the sixth edition of *Rheumatology*, amended with the section "Repair and regeneration of cartilage" by Linda J. Sandell.

REFERENCES

1. Heinegård D, Aspberg A, Franzén A, et al. Non-collagenous glycoproteins in the extracellular matrix, with particular reference to cartilage and bone. In: Royce P, Steinmann B, eds. *Connective tissue and its heritable disorders: molecular, genetic, and medical aspects*. New York: Wiley-Liss; 2002:271-291.

1A. Müller C, Khabut A, Dudhia J, et al. Quantitative proteomics at different depths in human articular cartilage reveals unique patterns of protein distribution. *Matrix Biol*. 2014;40:34-45.

2. Önnerfjord P, Khabut A, Reinholt FP, et al. Quantitative proteomic analysis of eight cartilaginous tissues reveals characteristic differences as well as similarities between subgroups. *J Biol Chem*. 2012;287:18913-18924.
3. Stattin EL, Wiklund F, Lindblom K, et al. A missense mutation in the aggrecan C–type lectin domain disrupts extracellular matrix interactions and causes dominant familial osteochondritis dissecans. *Am J Hum Genet*. 2010;86:126-137.
4. Glasson SS, Askew R, Sheppard B, et al. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature*. 2005;434:644-648.
5. Stanton H, Rogerson FM, East CJ, et al. ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro. *Nature*. 2005;434:648-652.
6. Sandy JD. A contentious issue finds some clarity: on the independent and complementary roles of aggrecanase activity and MMP activity in human joint aggrecanolysis. *Osteoarthritis Cartilage*. 2006;14:95-100.
7. Hughes CE, Caterson B, Fosang AJ, et al. Monoclonal antibodies that specifically recognize neoepitope sequences generated by "aggrecanase" and matrix metalloproteinase cleavage of aggrecan: application to catabolism in situ and in vitro. *Biochem J*. 1995;305:799-804.
8. Platt D, Bird JL, Bayliss MT. Ageing of equine articular cartilage: structure and composition of aggrecan and decorin. *Equine Vet J*. 1998;30:43-52.
9. Maroudas A, Bayliss MT, Uchitel-Kaushansky N, et al. Aggrecan turnover in human articular cartilage: use of aspartic acid racemization as a marker of molecular age. *Arch Biochem Biophys*. 1998;350:61-71.
10. Westling J, Fosang AJ, Last K, et al. ADAMTS4 cleaves at the aggrecanase site (Glu373-Ala374) and secondarily at the matrix metalloproteinase site (Asn341-Phe342) in the aggrecan interglobular domain. *J Biol Chem*. 2002;277:16059-16066.
11. Thomas L. Reversible collapse of rabbit ears after intravenous papain, and prevention of recovery by cortisone. *J Exp Med*. 1956;104:245-252.
12. Hardingham TE, Fitton-Jackson S, Muir H. Replacement of proteoglycans in embryonic chick cartilage in organ culture after treatment with testicular hyaluronidase. *Biochem J*. 1972;129:101-112.
13. Lorenzo P, Bayliss MT, Heinegård D. Altered patterns and synthesis of extracellular matrix macromolecules in early osteoarthritis. *Matrix Biol*. 2004;23:381-391.
14. Stubendorff JJ, Lammentausta E, Struglics A, et al. Is cartilage sGAG content related to early changes in cartilage disease? Implications for interpretation of dGEMRIC. *Osteoarthritis Cartilage*. 2012;20:396-404.
15. Blaschke UK, Eikenberry EF, Hulmes DJ, et al. Collagen XI nucleates self-assembly and limits lateral growth of cartilage fibrils. *J Biol Chem*. 2000;275:10370-10378.
16. Holmes DF, Kadler KE. The 10+4 microfibril structure of thin cartilage fibrils. *Proc Natl Acad Sci U S A*. 2006;103:17249-17254.
17. Aspden RM, Hukins DWL. Stress in collagen fibrils of articular cartilage calculated from their measured orientations. *Matrix*. 1989;9:486-488.
18. Lohmander LS, Atley LM, Pietka TA, et al. The release of crosslinked peptides from type II collagen into human synovial fluid is increased soon after joint injury and in osteoarthritis. *Arthritis Rheum*. 2003;48:3130-3139.
19. Gregory KE, Oxford JT, Chen Y, et al. Structural organization of distinct domains within the non-collagenous N-terminal region of collagen type XI. *J Biol Chem*. 2000;275:11498-11506.
20. Eyre DR. Collagens and cartilage matrix homeostasis. *Clin Orthop Relat Res*. 2004;427(suppl):S118-S122.
21. Vikkula M, Mariman ECM, Lui VCH, et al. Autosomal dominant and recessive osteochondrodysplasias associated with the *COL11A2* locus. *Cell*. 1995;80:431-437.
22. Vaughan L, Mendler M, Huber S, et al. D-periodic distribution of collagen type IX along cartilage fibrils. *J Cell Biol*. 1988;106:991-997.
23. Eyre DR, Pietka T, Weis MA, et al. Covalent cross-linking of the NC1 domain of collagen type IX to collagen type II in cartilage. *J Biol Chem*. 2004;279:2568-2574.
24. Fässler R, Schnegelsberg PNJ, Dausman J, et al. Mice lacking a1(IX) collagen develop noninflammatory degenerative joint disease. *Proc Natl Acad Sci U S A*. 1994;91:5070-5074.
25. Hedlund H, Mengarelli-Widholm S, Reinholt FP, et al. Stereologic studies on collagen in bovine articular cartilage. *APMIS*. 1993;101:133-140.
26. Maroudas A, Palla G, Gilav E. Racemization of aspartic acid in human articular cartilage. *Connect Tissue Res*. 1992;28:161-169.
27. Oldberg Å, Antonsson P, Lindblom K, et al. COMP (cartilage oligomeric matrix protein) is structurally related to the thrombospondins. *J Biol Chem*. 1992;267:22346-22350.
28. Kvansakul M, Adams JC, Hohenester E. Structure of a thrombospondin C-terminal fragment reveals a novel calcium core in the type 3 repeats. *EMBO J*. 2004;23:1223-1233.
29. Holmes DF, Gilpin CJ, Baldock C, et al. Corneal collagen fibril structure in three dimensions: structural insights into fibril assembly, mechanical properties, and tissue organization. *Proc Natl Acad Sci U S A*. 2001;98:7307-7312.
30. Shen Z, Heinegård D, Sommarin Y. Distribution and expression of cartilage oligomeric matrix protein and bone sialoprotein show marked changes during rat femoral head development. *Matrix Biol*. 1995;14:773-781.
31. Briggs MD, Chapman KL. Pseudoachondroplasia and multiple epiphyseal dysplasia: mutation review, molecular interactions, and genotype to phenotype correlations. *Hum Mutat*. 2002;19:465-478.
32. Svensson L, Aszodi A, Heinegård D, et al. Cartilage oligomeric matrix protein–deficient mice have normal skeletal development. *Mol Cell Biol*. 2002;22:4366-4371.
33. Saxne T, Månsson B, Heinegård D. Biomarkers for cartilage and bone in rheumatoid arthritis. In: Firestein G, Panayi G, Wollheim F, eds. *Rheumatoid arthritis: new frontiers in pathogenesis and treatment*. Oxford: Oxford University Press; 2006:301-313.
34. Scott PG, McEwan PA, Dodd CM, et al. Crystal structure of the dimeric protein core of decorin, the archetypal small leucine-rich repeat proteoglycan. *Proc Natl Acad Sci U S A*. 2004;101:15633-15638.
35. Scott PG, Dodd CM, Bergmann EM, et al. Crystal structure of the biglycan dimer and evidence that dimerization is essential for folding and stability of class I small leucine-rich repeat proteoglycans. *J Biol Chem*. 2006;281:13324-13332.
36. Kalamajski S, Aspberg A, Oldberg A. The decorin sequence SYIRIADTNIT binds collagen type I. *J Biol Chem*. 2007;282:16062-16067.
37. Danielson KG, Baribault H, Holmes DF, et al. Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. *J Cell Biol*. 1997;136:729-743.
38. Önnerfjord P, Heathfield TF, Heinegård D. Identification of tyrosine sulfation in extracellular leucine-rich repeat proteins using mass spectrometry. *J Biol Chem*. 2004;279:26-33.
39. Bengtsson E, Aspberg A, Heinegård D, et al. The amino-terminal part of PRELP binds to heparin and heparan sulfate. *J Biol Chem*. 2000;275:40695-40702.
40. Tillgren V, Önnerfjord P, Haglund L, et al. The tyrosine sulfate–rich domains of the LRR proteins fibromodulin and osteoadherin bind motifs of basic clusters in a variety of heparin-binding proteins, including bioactive factors. *J Biol Chem*. 2009;284:28543-28553.
41. Heathfield TF, Önnerfjord P, Dahlberg L, et al. Cleavage of fibromodulin in cartilage explants involves removal of the N-terminal tyrosine sulfate-rich region by proteolysis at a site that is sensitive to matrix metalloproteinase-13. *J Biol Chem*. 2004;279:6286-6295.
42. Danfelter M, Önnerfjord P, Heinegård D. Fragmentation of proteins in cartilage treated with interleukin-1: specific cleavage of type IX collagen by matrix metalloproteinase 13 releases the NC4 domain. *J Biol Chem*. 2007;282:36933-36941.
43. Bengtsson E, Mörgelin M, Sasaki T, et al. The leucine rich repeat protein PRELP binds perlecan and collagens and may function as a basement membrane anchor. *J Biol Chem*. 2002;277:15061-15068.
44. Rucci N, Rufo A, Alamanou MC, et al. The glycosaminoglycan-binding domain of PRELP acts as a cell type-specific NF-kappaB inhibitor that impairs osteoclastogenesis. *J Cell Biol*. 2009;187:669-683.
45. Lorenzo P, Aspberg A, Önnerfjord P, et al. Identification and characterization of asporin—a novel member of the leucine rich repeat protein family closely related to decorin and biglycan. *J Biol Chem*. 2001;276:12201-12211.
46. Kizawa H, Kou I, Iida A, et al. An aspartic acid repeat polymorphism in asporin inhibits chondrogenesis and increases susceptibility to osteoarthritis. *Nat Genet*. 2005;37:138-144.
47. Mustafa Z, Dowling B, Chapman K, et al. Investigating the aspartic acid (D) repeat of asporin as a risk factor for osteoarthritis in a UK Caucasian population. *Arthritis Rheum*. 2005;52:3502-3506.
48. Kalamajski S, Aspberg A, Lindblom K, et al. Asporin competes with decorin for collagen binding, binds calcium and promotes osteoblast collagen mineralization. *Biochem J*. 2009;423:53-59.
49. Couchman JR. Syndecans: proteoglycan regulators of cell-surface microdomains? *Nat Rev Mol Cell Biol*. 2003;4:926-937.
50. Bernfield M, Gotte M, Park PW, et al. Functions of cell surface heparan sulfate proteoglycans. *Annu Rev Biochem*. 1999;68:729-777.
51. Lamande SR, Shields KA, Kornberg AJ, et al. Bethlem myopathy and engineered collagen VI triple helical deletions prevent intracellular multimer assembly and protein secretion. *J Biol Chem*. 1999;274:21817-21822.
52. Wiberg C, Hedbom E, Khairullina A, et al. Biglycan and decorin bind close to the N-terminal region of the

collagen VI triple helix. *J Biol Chem*. 2001;276:18947-18952.
53. Wiberg C, Heinegård D, Wenglén C, et al. Biglycan organizes collagen VI into hexagonal-like networks resembling tissue structures. *J Biol Chem*. 2002;277:49120-49126.
54. Wiberg C, Klatt AR, Wagener R, et al. Complexes of matrilin-1 and biglycan or decorin connect collagen VI microfibrils to both collagen II and aggrecan. *J Biol Chem*. 2003;278:37698-37704.
55. Klatt AR, Becker AK, Neacsu CD, et al. The matrilins: modulators of extracellular matrix assembly. *Int J Biochem Cell Biol*. 2011;43:320-330.
56. Suleman F, Gualeni B, Gregson HJ, et al. A novel form of chondrocyte stress is triggered by a COMP mutation causing pseudoachondroplasia. *Hum Mutat*. 2012;33:218-231.
57. Wu JJ, Eyre DR. Matrilin-3 forms disulfide-linked oligomers with matrilin-1 in bovine epiphyseal cartilage. *J Biol Chem*. 1998;273:17433-17438.
58. Millward-Sadler SJ, Salter DM. Integrin-dependent signal cascades in chondrocyte mechanotransduction. *Ann Biomed Eng*. 2004;32:435-446.
59. Humphries MJ, Travis MA, Clark K, et al. Mechanisms of integration of cells and extracellular matrices by integrins. *Biochem Soc Trans*. 2004;32:822-825.
60. Haglund L, Tillgren V, Addis L, et al. Identification and characterization of the integrin alpha2beta1 binding motif in chondroadherin mediating cell attachment. *J Biol Chem*. 2011;286:3925-3934.
61. Haglund L, Tillgren V, Önnerfjord P, et al. The C-terminal peptide of chondroadherin modulates cellular activity by selectively binding to heparan sulfate chains. *J Biol Chem*. 2013;288:995-1008.
62. Pankov R, Yamada KM. Fibronectin at a glance. *J Cell Sci*. 2002;115:3861-3863.
63. Beauvais A, Erickson CA, Goins T, et al. Changes in the fibronectin-specific integrin expression pattern modify the migratory behavior of sarcoma S180 cells in vitro and in the embryonic environment. *J Cell Biol*. 1995;128:699-713.
64. Woods A, Longley RL, Tumova S, et al. Syndecan-4 binding to the high affinity heparin-binding domain of fibronectin drives focal adhesion formation in fibroblasts. *Arch Biochem Biophys*. 2000;374:66-72.
65. Homandberg GA, Kang Y, Zhang J, et al. A single injection of fibronectin fragments into rabbit knee joints enhances catabolism in the articular cartilage followed by reparative responses but also induces systemic effects in the non-injected knee joints. *Osteoarthritis Cartilage*. 2001;9:673-683.
66. Johnson A, Smith R, Saxne T, et al. Fibronectin fragments cause release and degradation of collagen-binding molecules from equine explant cultures. *Osteoarthritis Cartilage*. 2004;12:149-159.
67. Xu L, Peng H, Wu D, et al. Activation of the discoidin domain receptor 2 induces expression of matrix metalloproteinase 13 associated with osteoarthritis in mice. *J Biol Chem*. 2005;280:548-555.
68. Knudson CB, Knudson W, Smith RL. Hyaluronan and CD44: modulators of chondrocyte metabolism. *Clin Orthop Relat Res*. 2004;427(suppl):S152-S162.
69. Nedvetzki S, Gonen E, Assayag N, et al. RHAMM, a receptor for hyaluronan-mediated motility, compensates for CD44 in inflamed CD44-knockout mice: a different interpretation of redundancy. *Proc Natl Acad Sci U S A*. 2004;101:18081-18086.
70. Holmdahl R. Dissection of the genetic complexity of arthritis using animal models. *Immunol Lett*. 2006;103:86-91.
71. Sjöberg A, Önnerfjord P, Mörgelin M, et al. The extracellular matrix and inflammation: fibromodulin activates the classical pathway of complement by directly binding C1q. *J Biol Chem*. 2005;280:32301-32308.
72. Happonen KE, Sjöberg AP, Mörgelin M, et al. Complement inhibitor C4b-binding protein interacts directly with small glycoproteins of the extracellular matrix. *J Immunol*. 2009;182:1518-1525.
73. Happonen KE, Saxne T, Geborek P, et al. Serum COMP-C3b complexes in rheumatic diseases and relation to anti-TNF-alpha treatment. *Arthritis Res Ther*. 2012;14:R15.
74. Allen BL, Filla MS, Rapraeger AC. Role of heparan sulfate as a tissue-specific regulator of FGF-4 and FGF receptor recognition. *J Cell Biol*. 2001;155:845-858.
75. Gustafsson E, Aszodi A, Ortega N, et al. Role of collagen type II and perlecan in skeletal development. *Ann N Y Acad Sci*. 2003;995:140-150.
76. Kvist A, Johnson A, Mörgelin M, et al. Chondroitin sulfate perlecan enhances collagen fibril formation. Implications for perlecan chondrodysplasias. *J Biol Chem*. 2006;281:33127-33139.
77. Recklies AD, White C, Ling H. The chitinase 3–like protein human cartilage glycoprotein 39 (HC-gp39) stimulates proliferation of human connective-tissue cells and activates both extracellular signal–regulated kinase– and protein kinase B–mediated signalling pathways. *Biochem J*. 2002;365:119-126.
78. Thonar EJ, Feist SB, Fassbender K, et al. Quantification of hen egg white lysozyme in cartilage by an enzyme-linked immunosorbent assay. *Connect Tissue Res*. 1988;17:181-197.
79. Sharif M, Kirwan JR, Elson CJ, et al. Suggestion of nonlinear or phasic progression of knee osteoarthritis based on measurements of serum cartilage oligomeric matrix protein levels over five years. *Arthritis Rheum*. 2004;50:2479-2488.
80. Garnero P, Charni N, Juillet F, et al. Increased urinary type II collagen helical and C telopeptide levels are independently associated with a rapidly destructive hip osteoarthritis. *Ann Rheum Dis*. 2006;65:1639-1644.
81. Ameye LG, Deberg M, Oliveira M, et al. The chemical biomarkers C2C, Coll2-1, and Coll2-1NO2 provide complementary information on type II collagen catabolism in healthy and osteoarthritic mice. *Arthritis Rheum*. 2007;56:3336-3346.
82. Rai MF, Patra D, Sandell LJ, et al. Transcriptome analysis of human injured meniscus reveals a distinct phenotype of meniscus degeneration with aging. *Arthritis Rheum*. 2013;65(8).
83. Hangody L, Rathonyi GK, Duska Z, et al. Autologous osteochondral mosaicplasty. Surgical technique. *J Bone Joint Surg Am*. 2004;86-A(suppl 1):65-72.
84. Barber FA, Chow JC. Arthroscopic osteochondral transplantation: histologic results. *Arthroscopy*. 2001;17(8):832-835.
85. Solheim E, Hegna J, Oyen J, et al. Results at 10 to 14 years after osteochondral autografting (mosaicplasty) in articular cartilage defects in the knee. *Knee*. 2013;20(4):287-290.
86. Gortz S, Bugbee WD. Allografts in articular cartilage repair. *Instr Course Lect*. 2007;56:469-480.
87. Gross AE, Shasha N, Aubin P. Long-term followup of the use of fresh osteochondral allografts for posttraumatic knee defects. *Clin Orthop Relat Res*. 2005;435:79-87.
88. Grande DA, Pitman MI, Peterson L, et al. The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation. *J Orthop Res*. 1989;7(2):208-218.
89. Brittberg M, Lindahl A, Nilsson A, et al. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med*. 1994;331(14):889-895.
90. Colby SM, Hintermeister RA, Torry MR, et al. Lower limb stability with ACL impairment. *J Orthop Sports Phys Ther*. 1999;29(8):444-451, discussion 452-454.
91. Sterett WI, Hutton KS, Briggs KK, et al. Decreased range of motion following acute versus chronic anterior cruciate ligament reconstruction. *Orthopedics*. 2003;26(2):151-154.
92. McCarthy A, Kerr M, Abadie E, et al. Report on the joint EFPIA, DIA and EMEA pharmacogenetics workshop: moving toward clinical application. *Pharmacogenomics*. 2004;5(6):731-739.
93. Stanish WD, McCormack R, Forriol F, et al. Novel scaffold-based BST-CarGel treatment results in superior cartilage repair compared with microfracture in a randomized controlled trial. *J Bone Joint Surg Am*. 2013;95(18):1640-1650.
94. Minas T, Von Keudell A, Bryant T, et al. The John Insall Award: A minimum 10-year outcome study of autologous chondrocyte implantation. *Clin Orthop Relat Res*. 2014;472(1):41-51.
95. Wakitani S, Goto T, Pineda SJ, et al. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J Bone Joint Surg Am*. 1994;76(4):579-592.
96. Fellows CR, Matta C, Zakany R, et al. Adipose, bone marrow and synovial joint-derived mesenchymal stem cells for cartilage repair. *Front Genet*. 2016;7:213.
97. Barry FP, Murphy JM. Mesenchymal stem cells: clinical applications and biological characterization. *Int J Biochem Cell Biol*. 2004;36(4):568-584.
98. Barry F, Murphy M. Mesenchymal stem cells in joint disease and repair. *Nat Rev Rheumatol*. 2013;9(10):584-594.
99. Beitzel K, McCarthy MB, Cote MP, et al. Rapid isolation of human stem cells (connective progenitor cells) from the distal femur during arthroscopic knee surgery. *Arthroscopy*. 2012;28(1):74-84.
100. Gimble J, Guilak F. Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. *Cytotherapy*. 2003;5(5):362-369.
101. Bielli A, Scioli MG, Gentile P, et al. Adipose-derived stem cells in cartilage regeneration: current perspectives. *Regen Med*. 2016;11(7):693-703.
102. Bara JJ, Herrmann M, Evans CH, et al. Improving translation success of cell-based therapies in orthopaedics. *J Orthop Res*. 2016;34(1):17-21.
103. Boushell MK, Hung CT, Hunziker EB, et al. Current strategies for integrative cartilage repair. *Connect Tissue Res*. 2016;1-14.

3 Bone structure and function

David B. Burr • Teresita Bellido • Kenneth E. White

Key Points

- Bone is organized differently and for different functions at the organ, tissue, and molecular levels.
- Noncollagenous proteins function as structural support in the bone matrix and regulate mineral deposition, crystal growth, and cell attachment.
- Bone mineralization is effected through hormonal action (fibroblast growth factor 23 [FGF-23]) as well as through the bioactivity of intracellular and secreted matrix proteins with emerging roles (dentin matrix protein-1, FAM20c, and matrix extracellular phosphoglycoprotein).
- Growth factors, including insulin-like growth factor 1, the autocrine/paracrine FGFs, and transforming growth factor-β, play key roles in bone growth and structure.
- Osteoclasts are the primary bone-resorptive cells and originate from precursors of the hematopoietic lineage upon stimulation with receptor activator of nuclear factor-κB ligand (RANKL) and macrophage colony-stimulating factor.
- Whereas the rate of osteoclast generation determines the extension of the bone-remodeling unit, the life span of osteoclasts determines the depth of resorption.
- Osteoblasts are responsible for bone formation and originate from mesenchymal progenitors that also give rise to chondrocytes, muscle cells, and adipocytes.
- Osteocytes form a network that senses mechanical and hormonal environmental cues and orchestrates the function of osteoblasts and osteoclasts.
- Osteocytes produce and secrete factors (sclerostin/Sost, RANKL, osteoprotegerin) that affect other bone cells by paracrine/autocrine mechanisms and secrete hormones (FGF-23) that affect other tissues by endocrine mechanisms.
- Four dynamic processes—growth, modeling, remodeling, and repair—control skeletal development and adaptation, defined by the relationship of bone resorption and bone formation to each other.

INTRODUCTION

Bone is a complex natural composite material that undergoes millions of loading cycles during a lifetime without failure. Its structure is hierarchical, being organized differently at the organ (whole bone), tissue (material), and molecular (collagen–mineral) levels.

The mechanical functions of bone are the most widely recognized and are often described in terms of strength and stiffness. Although strength and stiffness are important, bone is particularly effective at dissipating energy that could cause a fracture over repeated cycles of loading. Beyond its mechanical function, the marrow cavity and the porous trabecular bone in the ends of long bones and in the vertebrae and iliac crest are regions in which red blood cells are formed and stored. Bone is in fact a primary blood-storing organ. Additionally, bone is the body's primary storehouse of calcium and phosphorus; 99% of the body's calcium is stored in bone. These minerals are necessary for the proper function of a variety of systems in the body and are essential for enzyme reactions, blood clotting, the proper function of contractile proteins in muscles, and the transmission of nerve impulses.

ORGANIZATION OF BONE

MACROSCOPIC (ORGAN) LEVEL

Bone at the organ level consists of the diaphysis (shaft), the metaphysis, and the epiphysis (Fig. 3.1). In the long bones of growing children, the growth plate separates the epiphysis from the metaphysis. The primary component of the diaphysis is cortical or compact bone. The haversian canals in cortical bone create a porosity of about 3% to 5%, although this increases in older age and with osteoporotic changes to the skeleton. Compact bone is also found surrounding the spongy bone of the vertebral body and in the skull. It is very strong and provides both support and protection.

Cancellous (trabecular or spongy) bone is found in the metaphyses of the long bones and in the vertebrae, surrounded by cortical bone. During growth, the primary spongiosa is composed mostly of disorganized woven bone, or primary lamellar bone surrounding a core of calcified cartilage. It is separated from the remodeled, more highly oriented secondary spongiosa by an arbitrary boundary. The secondary spongiosa reflects patterns of stress and functions largely to funnel stresses to the stronger and more massive cortical bone. In regions beneath joint surfaces, it also attenuates forces generated by mechanical loading and may protect the joint surface from loading-related trauma. The cancellous bone itself is composed of plates and rods of bone, each about 200 μm thick, with a porosity of about 75% to 85%. The marrow in the spaces between the trabecular struts are regions in which red blood cells are formed (red marrow). In osteoporosis, the differentiation of cells in the bone lineage can be partly diverted to adipocytes, and the marrow becomes more fatty (yellow marrow). Because cancellous bone has a large surface area in contact with vascular marrow, it is ideal for the long-term exchange of calcium ions. In osteopenia, regions of cancellous bone are affected first. This is why the vertebral column, which is mainly composed of cancellous bone, is affected earlier and more severely in osteoporosis than even the femoral neck and hip.

MICROSCOPIC (TISSUE) LEVEL

At the microstructural level, bone is organized differently for different functions (Fig. 3.2). In humans, bone tissue can be divided into three broad categories based partly on the arrangement of the collagen fibers and partly on whether it has replaced preexisting bone: (1) woven bone, (2) primary bone, and (3) secondary bone.

Woven bone is characterized by randomly oriented collagen fibers, which tend to be smaller in diameter than those in more highly organized primary or secondary bone. Woven bone is not lamellar, is very porous and may become highly mineralized. Woven bone can be deposited de novo without any bony or cartilaginous substrate or anlage, but it can also be formed as part of the process of endochondral ossification, either at the growth plate during development or during fracture repair. Woven bone proliferates rapidly because it has a large cell-volume ratio, which makes its role in fracture repair ideal. It is often found associated with osteosarcoma, probably because of proliferation especially of the cellular periosteum. The function of woven bone is primarily mechanical, rapidly providing both temporary strength and scaffolding upon which lamellar bone may be deposited. However, it can also be associated with pathologic processes that involve inflammatory cytokines.

Primary bone must be deposited on a preexisting substrate and is organized into lamellar layers. Because of this, trabecular plates, which are composed mainly of primary lamellae, cannot be replaced after they are perforated. This accounts for the loss of trabeculae with age and is part of the reason that it is difficult to reverse osteoporotic changes after trabecular connectivity has been lost. Primary lamellar bone also forms in rings around the endocortical and periosteal surfaces of whole bone (circumferential lamellae). Primary lamellar bone itself is not very vascular and can become very dense. Primary bone can also be arranged in concentric rings around a central canal—much like a secondary haversian system, except without a definable cement line. These primary osteons tend to be small with few concentric lamellae. In reality, primary osteons are modified vascular channels that have "filled in" by the addition of lamellae to the surface of the vascular space.

Secondary bone is the product of the resorption of preexisting bone and its replacement with new bone. This can occur within dense cortical bone (resulting in a secondary osteon, or haversian system) or begin on the surfaces of trabeculae (sometimes called a *hemi-osteon*). The distinction between primary and secondary bone is important because it is likely that control of primary bone apposition is different from replacement of preexisting bone by secondary bone. A secondary haversian system has a central vascular canal 50 to 90 μm in diameter. The blood vessels in the canal have the characteristics of capillaries and are generally paired within the canal. Venous sinusoids and lymphatic vessels are not found in haversian canals, although it has been suggested that prelymphatic vessels may exist.[1] The vessel walls

contain no smooth muscle but are fenestrated capillaries lined by an incomplete layer of endothelial cells, similar to vessels in other blood-forming organs like the spleen and bone marrow.[2] The vessel is accompanied about 60% of the time by two to seven unmyelinated or poorly myelinated nerve fibers.[3] Because the capillaries have no smooth muscle, these nerves do not serve a vasomotor function but are primary sensory nerves or autonomic nerves from the sympathetic nervous system. Both primary afferents and sympathetic nerve fibers express neuropeptides that may play some role in regulating bone remodeling. Afferent nerve fibers also express GAP43, which is associated with axonal growth, and may reflect the need for vessel growth and reinnervation during bone remodeling.

Lining cells (resting osteoblasts) cover the haversian canal, which is surrounded by a series of concentric lamellae containing bone cells—osteocytes. The relationship between the size of the osteon and the size of its canal is a measure of the balance between bone resorption (osteon diameter) and bone formation (canal diameter). The secondary osteon is bounded by a cement line, probably composed largely of sulfated glycosaminoglycans.[4] The cement line forms an effective boundary that can arrest cracks in bone and stop them from growing to a critical size.

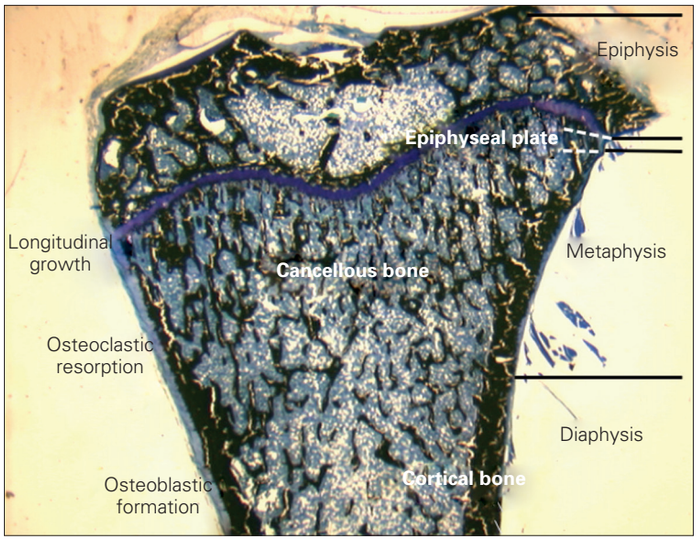

FIG. 3.1 Bone consists of the diaphysis (shaft), the metaphysis, and the epiphysis. In growing children, the growth (epiphyseal) plate separates the epiphysis from the metaphysis. During growth, the periosteal surface of the metaphysis must be constantly resorbed, while concurrent bone formation occurs on the endocortical surface to convert the thin-walled but flared metaphysis into the narrower but thicker-walled diaphysis (McNeal tetrachrome stain).

COMPOSITION OF BONE

Bone is composed of organic and mineralized components, mainly consisting of a matrix of cross-linked type I collagen mineralized with nanocrystalline, carbonated apatite. Bone matrix incorporates a small fraction of noncollagenous proteins that serve to control collagen assembly and size as well as the process of mineralization and cell attachment. The mineral comprises about 65% to 67% of the bone by weight, the organic component about 22% to 25%, and water the remaining fraction (≈10%). Within the organic fraction, type 1 collagen makes up about 90%, with the remainder accounted for by several minor collagens (types III and V) and a variety of noncollagenous proteins, most of them extracellular, with cell protein accounting for about 15% of the noncollagenous proteins in bone.

Type I collagen in bone is formed by a triple helix composed of two α1 chains and a single α2 chain. At either end of the collagen molecule are an N-telopeptide and a C-telopeptide, which can be cleaved when bone is resorbed and which are used to measure bone resorption biochemically. These individual triple helical collagen molecules self-associate into a periodic arrangement of parallel molecules spaced in quarter-staggered array at distances of 40 nm between their ends to form collagen fibrils (Fig. e3.1). The holes plus the overlap zones give the collagen its banded appearance with a D-periodic spacing of 67 nm on average. The spaces between the ends of the collagen molecules are known as *hole zones,* and the 35-nm gap zones that run longitudinally parallel between the molecules are known as *pores.* Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], which has small and poorly formed crystals, nucleates within these spaces. However, most of the mineral in bone, about 75%, is in the form of more highly carbonated apatite that forms plates about 300 nm long that lie outside the collagen fibril (extrafibrillar).[5,6] The initial deposition of mineral—or primary mineralization—occurs rapidly after new bone is deposited, with about two thirds of the eventual mineral content achieved within about 3 weeks.[7] As the bone tissue matures, the mineral crystals grow, become more platelike, and orient themselves parallel to one another and to the collagen fibrils. This process of crystal growth is sometimes called *secondary mineralization* and occurs over the next year or so until full mineralization is achieved. In cancellous bone, the c-axis or long axis of the mineral crystal aligns with the longitudinal axis of the trabeculae; in cortical bone, the mineral orients to the long axis of the osteon. Other elements can easily substitute into the hydroxyapatite crystal, changing its character and its ability to withstand mechanical loads. Collagen fibers can become cross-linked through the action of enzymes, which results in immature or reducible

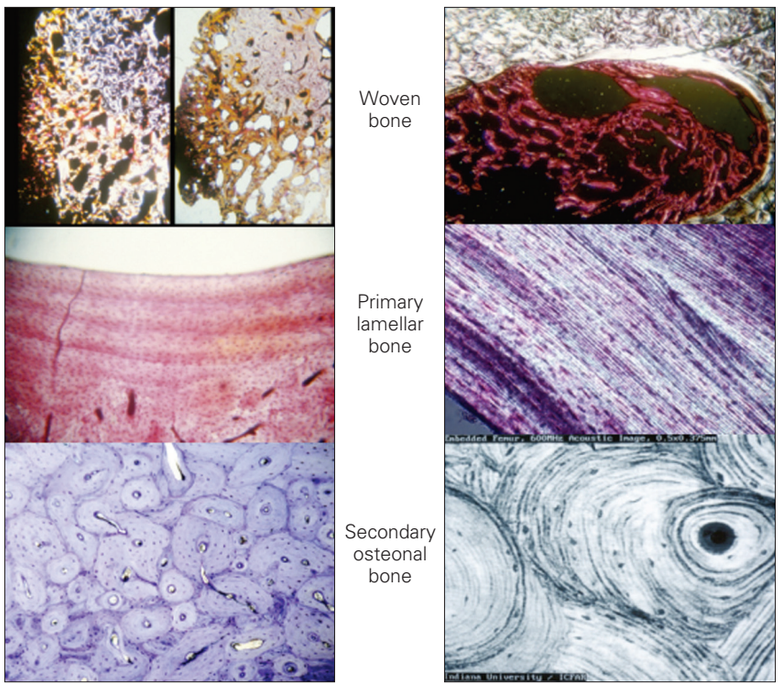

FIG. 3.2 The three general microscopic types of bone, defined morphologically. Woven bone is composed of disorganized, randomly oriented collagen fibers. It is found at sites of fracture or inflammation. Lamellar bone can be divided into primary and secondary. Primary bone is deposited in layers by direct apposition on a substrate. It is found circling the endocortical and periosteal circumferences of a long bone and within trabeculae. Secondary bone is formed by replacement of primary bone through the process of resorption and subsequent new bone formation. (Woven bone: polarized light. Primary lamellar bone: *left,* basic fuchsin; *right,* polarized light. Secondary osteonal bone: *left,* toluidine blue; *right,* scanning electron microscopic image.)

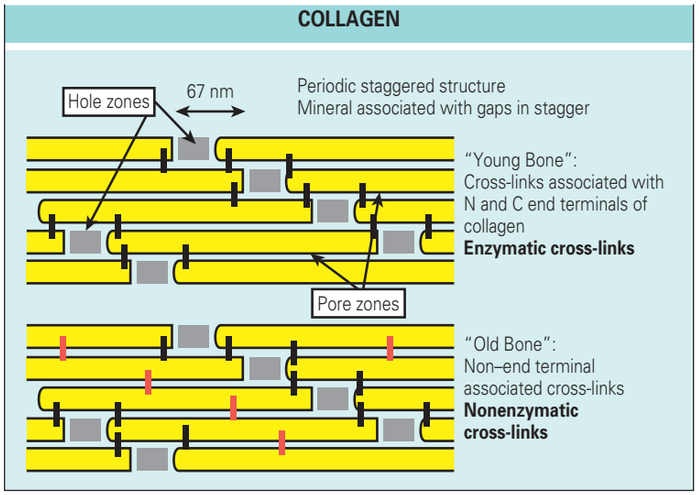

FIG. E3.1 In the collagen fibril, molecules are organized in a quarter-staggered array, with hole and pore zones between them where mineral can deposit. The hole zones give the collagen fibril its banded appearance when viewed by electron microscopy. Cross-links are formed either through the action of enzymes (divalent and trivalent cross-links) or through nonenzymatic processes, the latter forming advanced glycation end products that can make bone behave in brittle fashion.

(divalent) cross-links that mature through the action of lysyl oxidase to irreducible (trivalent) cross-links such as pyridinoline and deoxypyridinoline. Because divalent cross-links are rapidly converted into mature trivalent cross-links under normal circumstances, the quantity of divalent cross-links in bone is an indirect measure of remodeling rate. Divalent and trivalent cross-links are associated with the N- and C-terminals of the collagen molecule. Cross-links can also be formed between the fibers without the action of enzymes. These cross-links are formed by the condensation of arginine, lysine, and free sugars or through various oxidation reactions, which results in the formation of advanced glycation end products. Accumulation of advanced glycation end products occurs in diabetes, making the bone more brittle and increasing the risk of fracture.

The skeletal noncollagenous proteins comprise 10% to 15% of total bone protein. These molecules constitute a wide range of polypeptide species and have roles in multiple skeletal processes in addition to functioning as structural scaffolding within the bone matrix. One important feature of bone that has been largely overlooked until recently is water, which comprises about 10% of bone volume and can be found in either free-flowing or bound forms. Water stabilizes collagen structure[8] but also allows sliding at the interface between the mineral and the collagen, which increases the ductility and toughness of bone.[9] Because proteoglycans are hydrophilic, they may be important in retaining the water in some compartments.

PROTEOGLYCANS

Proteoglycans (PGs) are a ubiquitous family of molecules composed of a core protein and one or several covalently attached sulfated glycosaminoglycan chains. The glycosaminoglycans are linear polymers of repeated disaccharide units of hexosamine and hexuronic acid, except for keratan sulfate, in which hexuronic acid is replaced by galactose. The core proteins attached to the glycosaminoglycans are a diverse protein group and range in size from 10 to 500 kDa. The wide variety of protein structure may aid in directing the unique functional roles of each PG family.

The bone matrix contains PG families of several primary structures, including the following:

1. *Hyaluronan/CD44, chondroitin sulfate–containing PGs* are expressed in several regions of bone. Hyaluronan is expressed in focal regions within periosteum and endosteum and surrounding most of the major bone cell types including osteoblasts, osteoprogenitor cells, osteoclasts, and osteocyte lacunae. CD44 is a cell-surface hyaluronan receptor that may play roles in guiding bone development and has been localized to osteoclasts, osteocytes, and bone marrow cells.[10] Versican, a chondroitin sulfate–containing PG, may be enriched during early osteoid formation and may provide a temporary framework in newly formed cartilage matrix during bone development.[11] Results from cultured mesenchymal cells derived from a mouse model carrying a disrupted versican gene (the hdf transgenic mouse) also suggest that mature versican PG may play a role in directing limb chondrocyte aggregation depending on the surrounding extracellular matrix (ECM).[12]
2. *Heparan sulfate PGs* (HSPGs) are produced by osteoblast and osteoclast lineage cells. These molecules play important roles in cell–cell interactions during bone formation by trapping autocrine and paracrine heparin-binding fibroblast growth factor (FGF) family members, as well as acting as coreceptors with the FGF receptors. Also, other secreted molecules bind HSPGs, such as transforming growth factor-β (TGF-β, betaglycan) and osteoprotegerin (OPG) (syndecans). The bioactivity of these factors is modulated by HSPGs, potentially through focusing of concentrations of these potent molecules near differentiating cells.
3. *Small leucine-rich PGs* are the most abundant of the PGs in bone matrix and include decorin, biglycan, fibromodulin, lumican, and osteoadherin. These molecules help to provide the structural organization of the bone matrix and interact with specific growth factors and collagen to increase factor concentration and bioactivity in the matrix (Fig. 3.3). The localization of these proteins in mature bone varies; whereas decorin may localize with specific matrix areas, biglycan is evenly distributed throughout the matrix. Whereas decorin-null animals have alterations in collagen fibril size and bone shape,[13] biglycan-null animals demonstrate reduced bone mass because of lower osteoblast numbers and also show reduced numbers of osteoclasts.[14] Small leucine-rich PGs play an essential role in the regulation of growth factor activity. In this regard, decorin, biglycan, and fibromodulin all possess the ability to bind to TGF-β; however, decorin is the best characterized of these proteins for the ability to bind this factor. Decorin enhances TGF-β binding with its cognate receptors and enhances its bioactivity and, in concert, may act to sequester TGF-β in the collagen fibrils, thus reducing its activity. TGF-β activity is associated with increased apoptosis of osteoprogenitors; therefore, biglycan and decorin appear to be essential for maintaining mature osteoblast numbers through regulation of the proliferation and survival of bone marrow progenitor cells.

OSTEOCALCIN

Osteocalcin (OC) is a polypeptide posttranslationally modified to carry dicarboxylic glutamyl (Gla) residues, which relies on vitamin K for proper production (another identifier for OC is bone γ-carboxyglutamic acid [Gla] protein [BGLAP or BGP]). In humans, vitamin K is primarily a cofactor in the enzymatic reaction that converts glutamate residues into γ-carboxyglutamate residues in these vitamin K–dependent proteins including OC but also in proteins involved in blood clotting such as factor IX. These Gla-containing motifs are thought to enhance calcium binding, which may function to control mineral deposition and bone remodeling. A nine-residue domain proximal to the N-terminal of secreted OC shares high homology with the

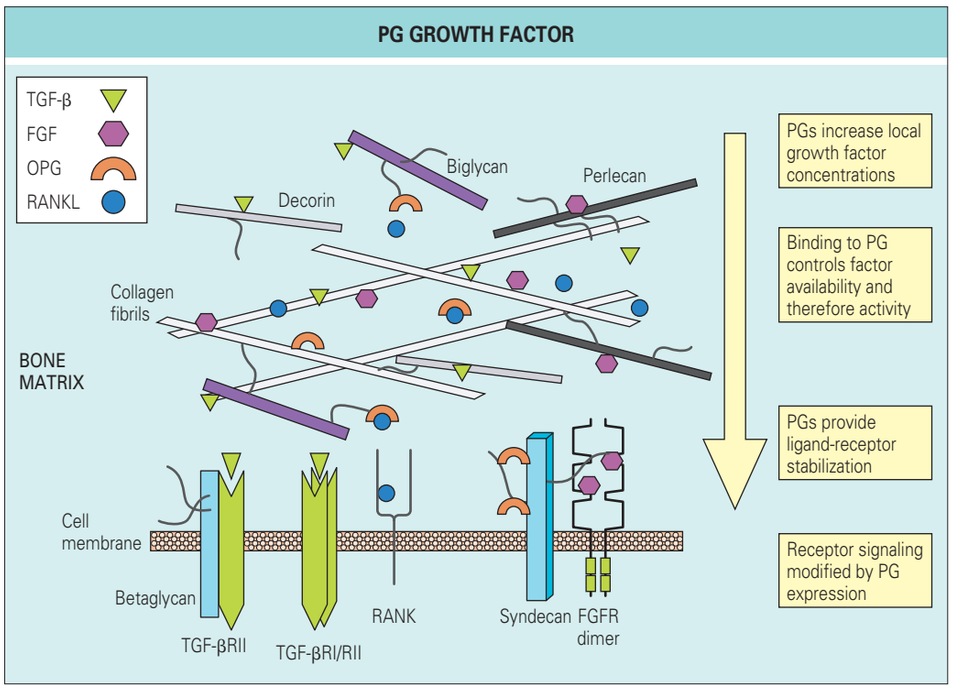

FIG. 3.3 Proteoglycans (PGs) regulate growth factors at several levels in the bone matrix and in bone cells. These polypeptides trap and locally concentrate endocrine and paracrine growth factors within the matrix. Soluble PGs such as decorin, biglycan, and perlecan also modulate activity through binding, thus controlling the concentrations of bioavailable factor. Membrane-bound PGs such as betaglycan and syndecan modulate ligand–receptor interactions and thus play roles in regulating intracellular signaling. *FGF,* Fibroblast growth factor; *FGFR,* fibroblast growth factor receptor; *OPG,* osteoprotegerin; *RANK,* receptor activator of nuclear factor-κB; *RANKL,* receptor activator of nuclear factor-κB ligand; *TGF-β,* transforming growth factor-β; *TGF-βRI/RII,* transforming growth factor-β receptor types I and II. *(Adapted from Lamoureux F, Baud'huin M, Duplomb L, et al. Proteoglycans: key partners in bone cell biology. Bioessays 2007;29:758-71.)*

corresponding regions in known propeptides of the γ-carboxyglutamic acid–containing blood coagulation factors. This common structural feature may be involved in the posttranslational targeting of these proteins for γ-carboxylation.

Osteocalcins may also act as a hormone to regulate the activity of osteoclasts and their precursors. In support of this, the skeleton of the OC-null animal manifests osteopetrosis compared with wild-type litter mates. In humans, OC is expressed largely by osteoblasts and osteocytes, and the measurement of this protein in serum has been used as a marker of bone turnover. OC messenger RNA (mRNA) is upregulated by vitamin D through interactions with *trans*-acting factors in vitamin D response elements in the OC promoter.[15] Because of its cell-specific expression, the OC promoter has proven to be invaluable as an active, functional DNA to drive foreign complementary DNAs in osteoblasts in transgenic animals.

OSTEOPONTIN

Osteopontin (OPN), also referred to as *secreted phosphoprotein-1,* is a member of the SIBLING (small integrin-binding ligand N-linked glycoprotein) family, which is a group of noncollagenous ECM proteins involved in bone mineralization. The genes coding for these proteins are localized to human chromosome band 4q21-25; have similar exon arrangements; and include those coding for dentin matrix protein-1, dentin sialoprotein, dentin phosphoprotein, integrin-binding sialoprotein, and matrix extracellular phosphoglycoprotein (MEPE). The SIBLING proteins share common structural features, such as multiple phosphorylation sites, a highly acidic nature, the presence of an arginine–glycine–aspartic acid cell attachment domain, and proteolysis-resistant acidic serine aspartate–rich MEPE-associated motif.

Osteopontin has a high sialic acid content and is produced by osteoblasts under stimulation by calcitriol. OPN is expressed within cement lines and may thus act as a promoter of adhesion and allow the arrangement of dissimilar tissues together in biologic composites such as teeth and bone.[16] OPN binds tightly to hydroxyapatite and may be involved in the anchoring of osteoclasts to the mineral of bone matrix. The vitronectin receptor, which has specificity for OPN, is focused within the osteoclast plasma membrane in the regions involved in the binding process. Long bones from OPN-null mice are indistinguishable from those from wild-type litter mates by radiography, but the relative amount of mineral in the more mature areas of the bone (central cortical bone) of the OPN-null mice is significantly increased, as is the mineral maturity (mineral crystal size and perfection) throughout all regions of the bone.[17] In vitro, exogenous OPN inhibits inorganic pyrophosphate (PP_i)–dependent mineralization of a cultured osteoblast cell line.[18] These findings indicate that OPN is a potent inhibitor of mineral formation as well as crystal growth and proliferation.

OSTEONECTIN

Osteonectin, also referred to as *secreted protein acidic and rich in cysteine* (SPARC), is a phosphoprotein that is the most abundant noncollagenous polypeptide expressed in bone. The mature protein binds selectively to hydroxyapatite, collagen fibrils, and vitronectin at distinct sites and may allow proper organization of the bone matrix through contacts with the cellular surface. Osteonectin also inhibits cellular proliferation through arrest of cells in the G1 phase of the cell cycle. It may regulate the activity of platelet-derived growth factor, vascular endothelial growth factor, and FGF-2. The osteonectin crystal structure has revealed a novel follistatin-like component and an extracellular calcium-binding region containing two EF-hand motifs. Osteonectin-null mice develop severe osteopenia, which indicates that this gene may have roles in osteoblast proliferation and in mineralization.

ALKALINE PHOSPHATASES AND ECTONUCLEOTIDE PYROPHOSPHATASE AND PHOSPHODIESTERASES

Alkaline phosphatases are widely distributed and are membrane-bound glycoproteins that hydrolyze monophosphate esters.[19] The liver–bone–kidney alkaline phosphatase, referred to as *tissue-nonspecific alkaline phosphatase* (encoded by the *ALPL* gene), acts as a lipid-anchored phosphoethanolamine and pyridoxal 5′-phosphate ectophosphatase. Loss-of-function mutations in the *ALPL* gene lead to hypophosphatasia, which is characterized by marked defects in bone mineralization and is lethal in the infantile form. The PP_i produced by cells inhibits mineralization by binding to crystals, and the presence of PP_i may thus prevent the soft tissues from undergoing mineralization. The degradation of PP_i to inorganic phosphate by ALPL in bones and teeth may facilitate crystal growth; therefore, it is thought that loss of function of the *ALPL* gene in hypophosphatasia results in accumulation of PP_i and reduced skeletal mineralization. Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) is a type II transmembrane glycoprotein and a member of the ENPP family. ENPP1 has broad specificity and cleaves phosphodiester bonds of nucleotides and nucleotide sugars as well as pyrophosphate bonds of nucleotides and nucleotide sugars. This protein may function to hydrolyze nucleoside 5′-triphosphates to their corresponding monophosphates and may also hydrolyze diadenosine polyphosphates. Loss-of-function mutations in ENPP1 result in autosomal recessive hypophosphatemic rickets type 2, characterized by a ricketic phenotype and elevation of FGF-23[20] (see later discussion).

THROMBOSPONDIN 1 AND 2

The thrombospondins (TSPs) are a family of secreted glycoproteins of high molecular mass. TSP1 and TSP2 share high homology and form 450-kDa homotrimers. Both TSP1 and TSP2 are expressed by mesenchymal cells and chondrocytes during cartilage formation. As osteoblasts replace the mineralizing cartilage, TSP2 expression decreases in chondrocytes and increases in the matrix within areas undergoing ossification. TSP1 and TSP2 are strong antiangiogenic factors and therefore may also play a role in controlling blood vessel organization in forming bone.

In developing animals, TSP1 and TSP2 are expressed in temporal and spatial patterns distinct for each gene. TSP1 (mouse gene, *Thbs1*) and TSP2 (*Thbs2*) have both been disrupted in mice and have unique phenotypes associated with each gene. Thbs1 is a regulator of TGF-β in vivo, and null animals have lower viability and prolonged wound healing. For skeletal phenotypes, this model has spine curvature and craniofacial alterations. Thbs2-null mice have increased cortical bone density, higher numbers of mesenchymal stem cells, and a resistance to bone loss due to ovariectomy. The fact that the Thbs2-null mice demonstrate less bone resorption than wild-type controls after ovariectomy may suggest a role for this molecule in estrogen-dependent reductions in bone mass and in the control of osteoclast function.

PROTEINS INVOLVED IN MINERALIZATION

Fibroblast growth factor 23

Fibroblast growth factor 23 is a phosphaturic hormone produced in bone (Fig. 3.4), and the encoding gene was identified as the mutated gene in

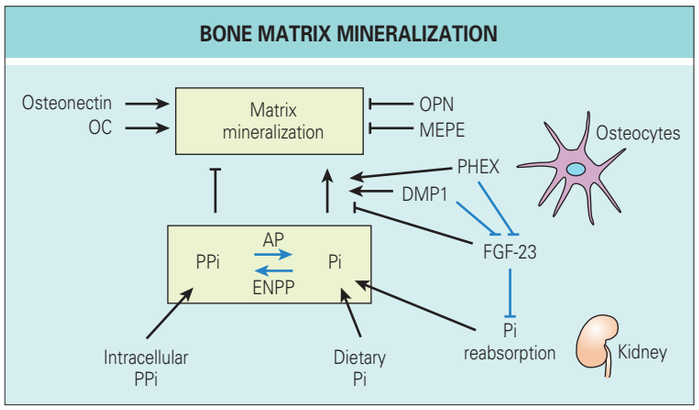

FIG. 3.4 Bone matrix mineralization involves an interplay of factors and is controlled by the balance between inorganic phosphate (P_i) and inorganic pyrophosphate (PP_i) and the expression of key local and systemic factors. Whereas an excess in P_i induces mineralization, PP_i inhibits it. Proteins expressed in osteocytes and osteoblasts regulate mineralization. Specifically, whereas PHEX (*p*hosphate-regulating gene with *h*omologies to *e*ndopeptidases on the *X* chromosome) and dentin matrix protein-1 (DMP1) induce mineralization, fibroblast growth factor 23 (FGF-23) inhibits mineralization. Inhibition of mineralization by FGF-23 is believed to be caused by inhibition of P_i reabsorption in the kidney, which reduces blood and bone P_i levels. Induction of mineralization by PHEX and DMP1 may be secondary to inhibition of FGF-23 and thus increases in circulating P_i. The P_i levels in the extracellular matrix depend on dietary intake and on its rate of synthesis from PP_i catalyzed by alkaline phosphatase (AP). Conversely, the levels of PP_i depend on its conversion from P_i by ectonucleotide pyrophosphatase/phosphodiesterase enzymes (ENPPs). Other bone-derived proteins, such as matrix extracellular phosphoglycoprotein (MEPE), osteopontin (OPN), osteocalcin (OC), and osteonectin, coordinately regulate mineralization, likely through direct interactions with the mineralized matrix. *(Adapted from Bellido T, Plotkin LI, Bruzzaniti A. Bone cells. In: Burr DB, Allen MR, editors. Basic and applied bone biology. San Diego: Academic Press; 2014, p. 27-46.)*

autosomal dominant hypophosphatemic rickets (ADHR), a metabolic bone disorder of isolated renal phosphate wasting.[21] Full-length FGF-23 (32 kDa) is the biologically active form of the protein and is inactivated upon cleavage into 20- and 12-kDa protein fragments. Intracellular cleavage of FGF-23 occurs between R_{179} and S_{180} within a highly charged subtilisin-like proprotein convertase (SPC) proteolytic site ($R_{176}H_{177}T_{178}R_{179}/S_{180}AE$). The human FGF-23 ADHR mutations R176Q, R179Q, and R179W destroy this site and stabilize the full-length active form of the protein. The production and secretion of whole-molecule, bioactive FGF-23 is a dynamic process. Within the trans-Golgi network, UDP-GalNAc transferase 3 (GALNT3) *O*-glycosylates FGF-23 on T_{180}. This glycosylation stabilizes bioactive FGF-23 by inhibiting cleavage between residues R_{179} and S_{180} via the SPC furin (PCSK3). The importance of this event is underscored by the fact that patients with GALNT3 inactivating mutations do not efficiently produce bioactive FGF-23.[22] The GALNT3-mediated FGF-23 glycosylation can be sterically hindered through prior phosphorylation of S_{180} by the novel kinase FAM20C,[23] thus providing a molecular interaction that controls the ability of osteoblasts and osteocytes to regulate serum phosphate concentrations and potentially bone mineralization (see Fig. 3.4). FGF-23 acts in the kidney to inhibit phosphate reabsorption by reducing expression of the proximal tubule type I sodium–phosphate cotransporters Npt2a[24] and Npt2c. The subsequent low serum phosphate level results in marked osteomalacia and rickets, fracture, and dental anomalies. Application of in situ hybridization to adult trabecular bone revealed the presence of FGF-23 mRNA in osteocytes and flattened bone-lining cells. In regions of active bone formation, newly formed osteocytes and osteoprogenitor cells also express FGF-23.[25] FGF-23 levels are elevated in vivo by increased serum phosphate and 1,25-dihydroxyvitamin D concentrations, and FGF-23 then completes the feedback loop by reducing phosphate reabsorption and 1,25-dihydroxyvitamin D production in the kidney. Evidence also indicates that FGF-23 is directly regulated by parathyroid hormone (PTH) in osteocytes.[26]

Whether FGF-23 has direct effects on the skeleton is uncertain because the FGF-23 coreceptor α-Klotho is predominantly expressed in the kidney and parathyroid glands. However, because FGF-23 is produced in bone, FGF-23 expression and its actions on serum phosphate concentrations may be coordinated with intraskeletal signals to allow proper bone formation and mineralization.

PHEX

X-linked hypophosphatemia, a disorder of rickets and osteomalacia, is caused by inactivating mutations in *PHEX* (*p*hosphate-regulating gene with *h*omologies to *e*ndopeptidases on the *X* chromosome).[27] PHEX encodes a protein that is similar to the M13 family of membrane-bound metalloproteases such as neutral endopeptidase and endothelin-converting enzymes 1 and 2 (see Fig. 3.4). These proteases are known to cleave small peptide hormones. Mutations in *PHEX* lead to dramatic over expression of FGF-23; however, the PHEX substrate and molecular mechanisms underlying this increase are currently unknown.

Dentin matrix protein-1

Dentin matrix protein-1 (DMP1), similar to OPN, is a member of the SIBLING gene family. DMP1 is highly expressed in osteocytes and is composed of 513 residues but is secreted in bone and dentin as 37-kDa N-terminal (residues 17–253) and 57-kDa C-terminal (residues 254–513) fragments from a 94-kDa full-length precursor (see Fig. 3.4). Recombinant DMP1 binds calcium-phosphate ions and the N-telopeptide region of type I collagen with high affinities, so in vivo DMP1 may regulate local mineralization processes in bone and teeth. The C-terminal portion of DMP1 has been implicated in DNA binding, in gene regulation, and as an integrin-binding protein. Inactivating mutations in DMP1 result in the metabolic bone disease autosomal recessive hypophosphatemic rickets, which is associated with elevated FGF-23 levels in these patients. As shown in the *Dmp1*-null mouse (and in the Hyp mouse model of X-linked hypophosphatemia), the primary cellular defect caused by loss of Dmp1 may be an alteration in osteoblast to osteocyte maturation, leading to inappropriate expression of typically "osteoblastic" or "early osteocyte" genes such as type I collagen, alkaline phosphatase, and FGF-23 in mature embedded osteocytes. The relationship of DMP1 to cell differentiation is currently unknown. Interestingly, FAM20c, a novel secreted kinase,[28] has been shown to phosphorylate the SIBLING proteins DMP1 and MEPE (see next section). Loss of FAM20c in mice results in a *Dmp* phenotype,[29] which indicates a complex relationship between the phosphorylation of ECM proteins and their normal functions.

Matrix extracellular phosphoglycoprotein

Another member of the SIBLING family found in the mineralizing matrix is MEPE (see Fig. 3.4). MEPE is predominantly expressed in odontoblasts and osteocytes embedded in the mineralized matrix. In vitro studies of human osteoblast cell cultures indicate that MEPE expression is the highest during the mineralization phase.[30] *Mepe*-null mice display increased trabecular and cortical bone mass because of increases in both osteoblast number and activity, and these mice are also resistant to age-dependent trabecular bone loss.[31] Taken together, these findings indicate that MEPE likely has a role as an important gene for the negative regulation of skeletal mineralization.

GROWTH FACTORS

Multiple growth factors, either produced within bone or circulating to bone, are critical for skeletal development and function. These factors may be sequestered within bone matrix via the bloodstream or may be produced by the major bone cell types and act as paracrine and autocrine factors.

Insulin-like growth factors

The insulin-like growth factors IGF-1 (somatomedin C) and IGF-2 (somatomedin A) are produced primarily in the liver but are also produced in bone. These factors predominantly circulate complexed with IGF binding proteins (IGFBPs) to facilitate their transport to tissues. IGFBPs can either enhance or inhibit IGF activity. IGF-1 and -2 act through the IGF-1 receptors (IGFR1 and IGFR2) and possess bioactivity that promotes cell proliferation and differentiation.

The IGF-1-null mouse has reduced cortical bone and femur length; however, trabecular density is increased. In vitro findings suggest that IGF-1 also increases osteoclastogenesis, and IGF-1-null mice have reduced levels of receptor activator of nuclear factor-κB ligand (RANKL) in osteoblasts isolated from bone marrow. Therefore IGF-1 may regulate osteoclastogenesis through direct and indirect actions. Overexpression of IGF-1 specifically in osteoblasts leads to increased bone mineral density and increased trabecular volume, although osteoblast numbers are not increased.[32] These studies suggest that IGF-1 acts directly on osteoblasts to enhance their function. Specific removal of IGFR1 from osteoblasts results in decreased trabecular number and volume and a dramatic decrease in bone mineralization, which further supports the role of the IGFs with regard to osteoblasts. Less is known regarding the functions of IGF-2 in bone. However, it has been suggested that IGF-2 may be a local regulator of bone cell metabolism.

Bone morphogenetic protein family

Bone morphogenetic proteins (BMPs) are members of the TGF-β superfamily. There are now more than 20 BMP-related proteins, which are classified into subgroups based on structure and function. These factors play important roles in skeletal development by directing the fate of mesenchymal cells, through differentiation of these precursor cells into cells of the osteoblastic lineage, and by inhibiting their differentiation into myoblastic lineage cells. BMPs also increase osteoclastogenesis, which is tightly coordinated with osteoblastogenesis. BMPs activate specific receptors and induce cell signaling by phosphorylating cytoplasmic receptor–regulated Smads, which enter the nucleus to recruit transcription factors and enhance gene expression. The human disorder fibrodysplasia ossificans progressiva is a disease of dramatic ectopic bone formation, which can be accelerated after blunt trauma. A recurrent mutation in activin receptor IA/activin-like kinase 2, a BMP type I receptor, was reported as the molecular cause of fibrodysplasia ossificans progressiva.[33] These findings underscore the potent effects of BMP signaling on bone formation.

Fibroblast growth factors

Members of the FGF family of proteins primarily act as paracrine and autocrine factors and bind to one or several of four FGF receptors (FGFRs). FGFRs normally exist as an inactivated monomer. With FGF binding in the presence of heparin/heparan sulfate, the FGFRs dimerize, which leads to the autophosphorylation of tyrosine residues. The FGF family has potent effects on bone development. This is clearly evident by the fact that activating mutations in FGFR1 and FGFR2 are responsible for disorders of craniosynostosis and limb patterning, and FGFR1 and FGFR3 mutations result in disorders of hypochondroplasia and achondroplasia. The FGFs interact with HSPGs and are sequestered within the mineralizing matrix. In addition, the HSPG syndecan may stabilize FGF-FGFR interactions and promote FGF signaling and bioactivity.

The FGF family members play important roles in bone development and formation. Expression of several FGF ligands, including FGF-2, FGF-5, FGF-6, FGF-7, and FGF-9, has been observed in mesenchyme surrounding the initial congregations of cells that proliferate and differentiate to form bone. In limb bud, FGFR1 and FGFR2 are expressed in condensing mesenchyme. In rat growth plates, mRNAs encoding all four FGFRs and FGF-2 can be detected, and FGF-2 is also present in osteoblasts. FGF-2 treatment

of osteoblasts enhances the binding of Runx2 to the Cbfa1 consensus sequence in the OC promoter and may therefore have a role in differentiation.

Transforming growth factor-β

Transforming growth factor-β controls proliferation, differentiation, and other functions in many cell types. TGF-β1, TGF-β2, and TGF-β3 all function through the type I and type II TGF receptors. The type I TGF-β receptor forms a heterodimer with the type II TGF-β receptor. TGF-β stimulation leads to activation of SMAD2 and SMAD3, which form complexes with SMAD4 that accumulate in the nucleus and regulate the transcription of target genes.

Transforming growth factor-β is the most abundant growth factor in human bone; it is localized within the bone matrix and has functions both during embryonic development and in mature bone. During embryonic development, TGF-β1 plays a role in cell migration, controlling epithelial–mesenchymal interactions, and the formation of cellular condensations, which influence bone shape. This factor also plays a key role in inducing mesenchymal cell differentiation to either chondrocytes or osteoblasts. In adult bone, TGF-β1 controls osteoblast differentiation, which affects matrix formation and mineralization. TGF-β1 inhibits the expression of the differentiation markers Runx2 and OC in osteoblast cell lines, and its functions interplay with those of other systems in bone such as the PTH and the Wnt/β-catenin systems.

The skeletal disorder Camurati-Engelmann disease (CED) highlights the importance of TGF-β1 in skeletal formation. CED is a progressive diaphyseal dysplasia characterized by hyperostosis and sclerosis of the diaphyses of the long bones. The *TGFB1* gene was screened and three different heterozygous missense mutations were found in exon 4 in the nine families examined. All of the mutations in the CED patients were located either at C225 or near R218, which suggests the importance of this region in activating TGF-β1 in the bone matrix.

Platelet-derived growth factor and vascular endothelial growth factor

All platelet-derived growth factors (PDGFs) and vascular endothelial growth factors (VEGFs) are dimers of disulfide-linked polypeptide chains, encoded by nine different genes that direct production of four different PDGF chains (PDGF-A, PDGF-B, PDGF-C, and PDGF-D) and five different VEGF chains (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor). All members of these families carry a growth factor core domain that is necessary for receptor activation. PDGFs mediate their bioactivity through two receptors, PDGFR-α and PDGFR-β. These receptors both have five extracellular immunoglobulin loops for ligand binding and an intracellular tyrosine kinase domain. The VEGFs act through a homologous family of receptors, VEGFR1, VEGFR2, and VEGFR3. PDGFs act primarily as paracrine growth factors.

Platelet-derived growth factor is chemotactic and mitogenic for osteoblasts and osteoprogenitor cells, and it upregulates cytokines that are crucial to bone healing. This factor also destabilizes blood vessels during healing to allow sprouting of new vessels. VEGF is produced by many cell types including fibroblasts, hypertrophic chondrocytes, and osteoblasts. VEGF may act not only in bone angiogenesis and vascular differentiation but also in aspects of development, such as chondrocyte and osteoblast differentiation, as well as osteoclast recruitment.

BONE CELLS

Bone development and the adaptation of the adult skeleton to mechanical needs and hormonal changes depend on the ability of bone cells to resorb and form bone in the right places and at the right time. Bone growth, modeling, and remodeling are defined by the spatial and temporal relationship between bone resorption and bone formation. Osteoclasts resorb bone, osteoblasts form bone, and osteocytes detect the need for bone augmentation or reduction and coordinate the activity of osteoclasts and osteoblasts.

OSTEOCLASTS

Osteoclasts are the primary bone-resorptive cells. They are needed for bone modeling, which leads to changes in the shape of bones, and for bone remodeling, which maintains the integrity of the adult skeleton. Osteoclasts originate from precursors of the monocyte/macrophage family of the hematopoietic lineage that differentiate to multinucleated cells upon stimulation with RANKL and macrophage colony-stimulating factor (M-CSF) (Fig. 3.5). Upon completing bone resorption, all osteoclasts undergo programmed cell death or apoptosis and disappear from the bone surface.

Osteoclast morphology and function

Osteoclasts adhere firmly to bone through the interactions established between integrins expressed in the osteoclast membranes with collagen, fibronectin, and other bone matrix proteins. Expression of α_V and α_3 integrin is induced during osteoclast differentiation, and the integrin binds to the amino acid sequence Arg-Gly-Asp present in OPN and bone sialoprotein. The importance of these events for osteoclast activity is underscored by the inhibition of resorption with competitive Arg-Gly-Asp ligands[34] and a progressive increase in bone mass caused by osteoclast dysfunction in mice null for β_3 integrin.

The intimate contact between the osteoclast and the bone matrix creates a space called the *sealing zone*. There is also polarization of the osteoclast fibrillar actin into a circular structure called the *actin ring,* containing podosomes composed of an actin core surrounded by $\alpha_V\beta_3$ integrins and associated cytoskeletal and signaling proteins. Thus, the area in which the osteoclast apposes the bone is isolated from the general extracellular space and becomes acidified by the activity of a proton pump and a chloride channel.[34] The low pH in this area dissolves the mineral and exposes the organic matrix, which is subsequently degraded by the activity of lysosomal cathepsin K and matrix metalloproteases. These degrading enzymes are

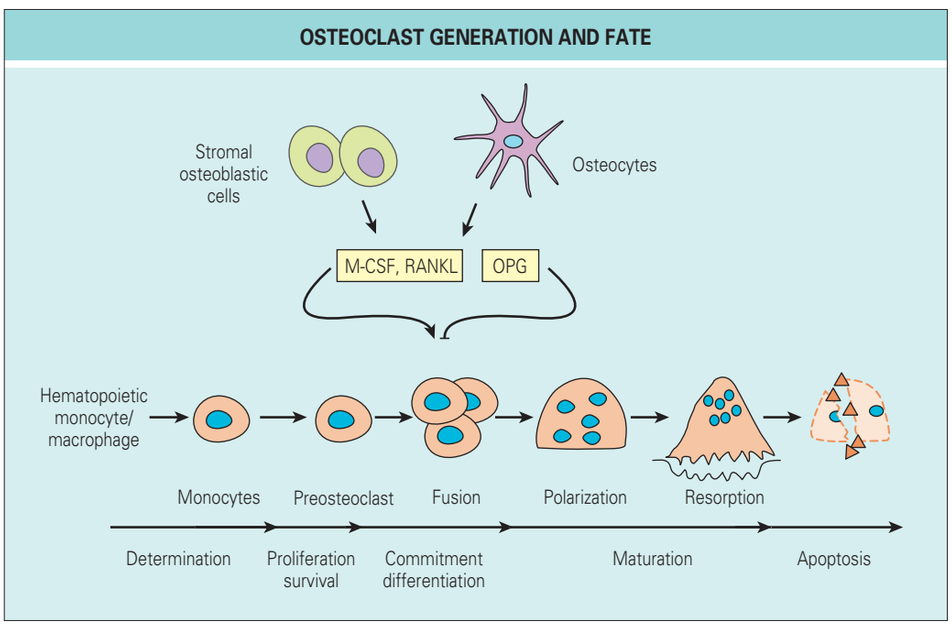

FIG. 3.5 Osteoclast differentiation is governed by receptor activator of nuclear factor-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) secreted by osteoblasts and osteocytes, which control various steps of the osteoclast differentiation process, including precursor proliferation, commitment, differentiation, and maturation. Osteoprotegerin (OPG), which is also secreted by osteoblasts and osteocytes, acts as a decoy receptor for RANKL and reduces osteoclast differentiation. *(From Bellido T, Plotkin LI, Bruzzaniti A. Bone cells. In: Burr DB, Allen MR, editors. Basic and applied bone biology. San Diego: Academic Press; 2014, p. 27-46.)*

transported into acidified vesicles that fuse with the osteoclast plasmalemma, forming a villous structure referred to as the *ruffled border.* This structure and the actin ring are essential features of a resorbing osteoclast, and abnormalities of either structure lead to arrested bone resorption.

The cytoplasmic domains of integrins serve as platforms for signaling proteins involved in osteoclast function, such as the kinase Src, which is crucial for osteoclast attachment and resorption. Src regulates podosome disassembly and ruffled membrane formation by its ability to interact with the focal adhesion–related kinase Pyk2 and the proto-oncogene c-Cbl. Rho, Rac, and the guanine nucleotide exchange factor Vav3, which activates guanosine diphosphatases into guanosine triphosphatases, also play a central role in modifying the resorptive capacity of osteoclasts by modulating the actin cytoskeleton. Osteoclast resorption products are transported in vesicles through the cytosol to the basolateral surface and discharged to the extracellular milieu or directly released to the surrounding fluid after osteoclast retraction from the resorption pits.

Osteoclast formation and differentiation

Mature, multinucleated osteoclasts are formed by fusion of mononuclear precursors of the monocyte/macrophage lineage (see Fig. 3.5). The earliest recognized osteoclast precursor is the granulocyte-macrophage colony-forming unit, which also gives rise to granulocytes and monocytes. Osteoclast precursors proliferate in response to growth factors such as interleukin-3 (IL-3) and colony-stimulating factors like granulocyte-macrophage colony-stimulating factor (GM-CSF) and M-CSF to form postmitotic, committed mononucleated osteoclast precursors, which differentiate and fuse to form multinucleated osteoclasts under the influence of RANKL, a member of the tumor necrosis factor (TNF) family of ligands.

M-CSF and RANKL are critical for osteoclastogenesis, and deletion of M-CSF, RANKL, or RANK (the receptor for RANKL expressed by osteoclasts and their precursors) inhibits osteoclast differentiation, leading to osteopetrosis in mice. Both M-CSF and RANKL are expressed by bone marrow stromal cells and osteoblastic cells, as well as T lymphocytes and other cell types in pathologic settings. Importantly, osteocytes have now been found to be a major source of M-CSF and RANKL, as well as OPG, the RANKL decoy receptor,[35,36] and deletion of RANKL from osteocytes leads to osteopetrosis,[36] demonstrating a central role of osteocytes in osteoclastogenesis (see Fig. 3.5). Whereas M-CSF contributes to osteoclast differentiation, migration, and survival by binding to its receptor c-Fms on osteoclast precursors, RANKL facilitates osteoclast formation via direct binding to the receptor RANK. RANKL is expressed on the cell surface and is also secreted as a soluble form. Although the soluble form of RANKL is found in the circulation and its presence is sufficient to induce differentiation of osteoclast precursors in vitro, its actual role in osteoclast formation in vivo remains unproven.

RANKL expression is upregulated by hormones and cytokines known to induce osteoclast generation. This explains the long-observed property of primary osteoblastic cells or osteoblastic cell lines that, upon treatment with vitamin D, PTH, or IL-11, IL-6, TNF, and IL-1, support osteoclast development when co-cultured with osteoclast precursors derived from spleen or bone marrow. RANKL mediates several aspects of osteoclast differentiation, including fusion of mononucleated precursors into multinucleated cells, acquisition of osteoclast-specific markers, attachment of osteoclasts to the bone surfaces, stimulation of resorption, and promotion of osteoclast survival. Although M-CSF contributes to RANKL effects, RANKL appears to play a dominant role in bone resorption. Thus, whereas M-CSF–null mice recover with time from the decreased osteoclast number and activity, RANKL knockout mice do not. Furthermore, RANKL appears to stimulate osteoclast formation and resorption in mice even in the absence of functional M-CSF.[37]

RANKL activates several signal transduction pathways involving the recruitment of the adapter protein TRAF6 (TNF receptor–associated factor 6) to the intracellular domain of the receptor RANK. TRAF6 in turn activates kinase-dependent signaling as well as transcription factors. Among them, NF-κB has been shown to undergo nuclear translocation, leading to upregulation of c-Fos. c-Fos, in turn, binds to nuclear factor of activated T cells, cytoplasmic 1 (NFATc1) and upregulates genes crucial for osteoclast differentiation and function. Although other signaling pathways are activated by RANKL in osteoclasts, the evidence that deletion of NF-κB, c-fos/AP1, and NFATc1 leads to osteoclast dysfunction demonstrates the crucial role of these genes in osteoclasts.[37]

Osteoprotegerin is an inhibitor of RANK activation and osteoclastogenesis that also belongs to the TNF family of receptors. OPG is a secreted protein with no transmembrane domain, and therefore it has no direct signaling capabilities. OPG suppresses osteoclast formation and resorption by binding to RANKL, thereby impeding RANKL interaction with RANK.

Osteoclast apoptosis

All osteoclasts undergo apoptosis and disappear from the bone surface after completing bone resorption. High concentrations of extracellular calcium, similar to the ones present in resorption cavities, induce osteoclast apoptosis in vitro and may be the triggering event. Fas ligand stimulates osteoclast apoptosis, and Fas-deficient mice exhibit more osteoclasts and decreased bone mass, which suggests that this pathway controls osteoclast life span in vivo. Osteoclast apoptosis might also result from loss of survival signals provided by integrin interactions with the matrix or by changes in the production of cytokines or growth factors that preserve osteoclast viability. Potential antiapoptotic factors are M-CSF and RANKL, the same cytokines that induce osteoclast differentiation. TNF-α and IL-1 also delay osteoclast apoptosis. All of these cytokines activate the extracellular signal–regulated kinases (ERKs), the activation of which is required for osteoclast survival. Phosphatidylinositol 3′-kinase (PI3-K) and its target the kinase Akt are required for osteoclast differentiation but not for survival. Instead, mammalian target of rapamycin (mTOR), another PI3-K target, is required for the antiapoptotic actions of M-CSF, RANKL, and TNF-α in osteoclasts. Because mTOR is also activated by ERKs, it appears to be a point of convergence in the action of prosurvival kinases in osteoclasts.

RANKL, TNF-α, and IL-1 also activate NF-κB, a transcription factor shown to inhibit apoptosis in various cell types. Downregulation of NF-κB mRNA inhibits IL-1–dependent survival, and blockade of NF-κB binding to DNA with specific oligonucleotides induces apoptosis. However, osteoclast precursors lacking NF-κB subunits have normal survival rates, and inhibition of NF-κB activation via a dominant-negative IKK2 does not affect the ability of IL-1 to promote osteoclast survival. Therefore, the relevance of NF-κB signaling for osteoclast survival is still controversial.

Regulation of osteoclast generation and survival

In the bone-remodeling unit, whereas the rate of osteoclast generation determines the extension of the bone-remodeling unit, the life span of osteoclasts determines the depth of resorption. Although both genesis and apoptosis of osteoclasts lead to changes in osteoclast number and bone resorption, alteration of osteoclast life span might represent a more effective mechanism to accomplish rapid changes in bone resorption rate.

Sex steroids have profound effects on osteoclasts. Both estrogens and androgens inhibit osteoclast generation by regulating the production of pro-osteoclastogenic cytokines (e.g., IL-6 and IL-1) by cells of the stromal/osteoblastic lineage. Estrogens also induce apoptosis of mature osteoclasts. This, together with an inhibitory effect of the hormones on osteoblast generation, leads to attenuation of the rate of bone remodeling.

Mice receiving excess glucocorticoids exhibit reduced osteoclast progenitors, but cancellous osteoclast number does not decrease in the early phases of the disease because glucocorticoids prolong the life spans of preexisting osteoclasts. This effect may account for the early transient increase in bone resorption in patients with hyperglucocorticoidism. In contrast to the rapid prosurvival effect of glucocorticoids on mature osteoclasts, glucocorticoids induce a decrease in osteoclast formation caused by a reduction in the pool of osteoblastic cells that support osteoclastogenesis. This effect leads to the typical low remodeling rate observed in chronic glucocorticoid-induced osteoporosis.

OSTEOBLASTS

Osteoblasts are the cells responsible for bone formation. They originate from mesenchymal progenitors, which also give rise to chondrocytes, muscle cells, and adipocytes (Fig. 3.6). Commitment of mesenchymal cells to the osteoblastic lineage depends on the specific activation of transcription factors induced by morphogenetic and developmental proteins that carry out the functions of bone matrix protein secretion and bone mineralization. Upon completion of bone matrix formation, some mature osteoblasts remain entrapped in bone as osteocytes, some flatten to cover quiescent bone surfaces as bone-lining cells, and most die by apoptosis.

Osteoblast function

The main function of osteoblasts is to synthesize collagen type I and other specialized matrix proteins that serve as a template for the subsequent mineral deposition in the form of hydroxyapatite. Mature osteoblasts actively engaged in this process are recognized by their location on the bone surface and by their morphologic features typical of cells secreting high levels of proteins: cuboidal shape with large nucleus, enlarged Golgi apparatus, and extensive endoplasmic reticulum. Osteoblasts express high levels of alkaline phosphatase and OC, and the level of these proteins in blood reflects the rate of bone formation.

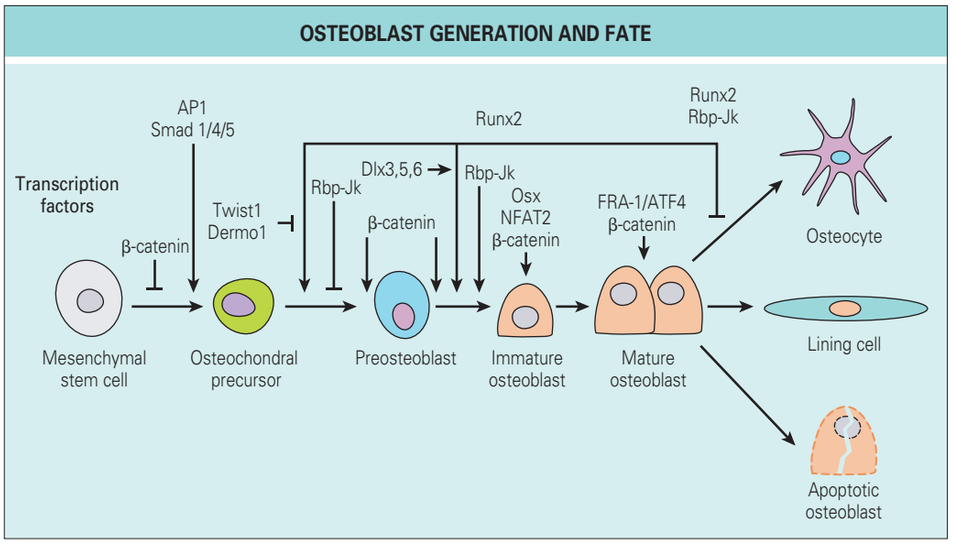

FIG. 3.6 Osteoblastogenesis is controlled by transcription factors that affect the proliferation and differentiation of osteoblast precursors. Mature osteoblasts can surround themselves by bone matrix and differentiate further to become osteocytes, flatten to cover the quiescent bone surface as lining cells, or die by apoptosis. *(From Bellido T, Plotkin LI, Bruzzaniti A. Bone cells. In: Burr DB, Allen MR, editors. Basic and applied bone biology. San Diego: Academic Press; 2014, p. 27-46.)*

Interaction of osteoblasts among themselves, with lining cells, and with bone marrow cells is established by adhesion junctions, tight junctions, and gap junctions. Adhesion junctions mainly mediated by cadherins and tight junctions serve to join cells and facilitate their anchorage to the ECM. Changes in the expression level of the major cadherins expressed in osteoblasts, N-cadherin and cadherin 11, influence osteoblast differentiation and survival. Intercellular communication among osteoblasts and neighboring cells is maintained by cell coupling via gap junctions. Opening of gap junction channels contributes to coupling and the coordination of responses within a cell population. The major gap junction protein expressed in bone cells is connexin 43. Its absence or dysfunction leads to impaired osteoblast differentiation, premature apoptosis of osteoblasts and osteocytes, and deficient response to hormones and pharmacotherapeutic agents.[38] Furthermore, gap junction communication is fundamental for the maintenance of a continuum from bone, where osteocytes reside, through bone surface cells, osteoblasts and osteoclasts, bone marrow cells, and endothelial cells of the blood vessels.[39] This functional syncytium might be responsible for the coordinated response of the bone tissue to changes in physical and chemical stimuli, as will be discussed later. Interactions between osteoblasts and the bone matrix via integrins also modulate osteoblast differentiation, function, and survival. In particular, loss of antiapoptotic signals provided by the ECM causes apoptosis, a phenomenon referred to as *anoikis*.

Osteoblast formation and differentiation

The process of osteoblastogenesis can be divided into steps comprising proliferation, ECM development and maturation, mineralization, and apoptosis. Each stage is characterized by activation of specific transcription factors and genes leading to a succession of osteoblast phenotypic markers (see Fig. 3.6). Transcription factors of the helix-loop-helix family (Id, Twist, and Dermo) are expressed in proliferating osteoblast progenitors and are responsible for maintaining the osteoprogenitor population by inhibiting the expression of genes that characterize the osteoblast mature phenotype. Transcription factors of the activating protein family, such as c-fos, c-jun, and junD, are expressed during proliferation as well as later in the differentiation pathway and may activate or repress transcription. Runx2 and osterix are essential for establishing the osteoblast phenotype. Their absence from the mouse genome results in lack of skeletal mineralization and perinatal lethality. Runx2 and osterix regulate the expression of other genes that control bone formation and remodeling, including OC and RANKL. Runx2 regulates differentiation, survival, and function of osteoblasts by affecting several signaling pathways, including those activated by Wnts, BMPs, integrins, and the PTH receptor.

Osteoblast apoptosis

Upon completing the process of bone formation, 60% to 70% of osteoblasts die by apoptosis; the rest become lining cells or osteocytes. Apoptosis occurs throughout all stages of osteoblast life.[40] The prevalence of osteoblast apoptosis in bone sections can be quantified by measuring fragmented DNA. Apoptosis of cultured osteoblasts has been extensively studied using several methods,[41] including increased activity of initiator or effector caspases, the presence of cleaved genomic DNA by TUNEL or ISEL assay, and nuclear fragmentation and chromatin condensation using fluorescent dyes that bind to DNA. Examination of the nuclear morphology of cells transfected with fluorescent proteins containing a nuclear localization sequence has proven a particularly useful tool for studying apoptosis in cells co-transfected with genes of interest. Cell detachment from the substrate, changes in the composition of the plasma membrane, and changes revealing cell shrinkage are also features that have been used to detect and quantify apoptotic cells.

Regulation of osteoblast generation and apoptosis

Most major regulators of skeletal homeostasis influence both generation and survival of osteoblasts. The BMP and Wnt signaling pathways promote osteoblast differentiation, but whereas BMPs induce osteoblast apoptosis, Wnts inhibit it. BMPs induce apoptosis of mature osteoblasts as well as of mesenchymal osteoblast progenitors in interdigital tissues during the development of the hands and feet. Wnt signaling has a profound effect on bone as shown by the high-bone-mass phenotype of mice and humans with activating mutations of low-density lipoprotein receptor–related protein 5 (LRP5), which together with Frizzled proteins are receptors for Wnt ligands. Wnts stimulate differentiation of undifferentiated mesenchymal cells toward the osteoblastic lineage and stimulate differentiation of preosteoblasts. Canonical Wnt signaling in osteoblasts also affects osteoclasts by enhancing the expression of the RANKL decoy receptor OPG, which leads to inhibition of osteoclast development. In addition, Wnt signaling inhibits apoptosis of mature osteoblasts and osteocytes.[42] The increased bone formation exhibited by mice lacking the Wnt antagonist known as secreted Frizzled related protein-1 (sFRP-1) is associated with decreased osteoblast and osteocyte apoptosis. The prevalence of osteoblast and osteocyte apoptosis is also decreased in mice expressing the high bone mass–activating mutation of LRP5 (G171V), which exhibit reduced ability to bind the Wnt antagonist sclerostin secreted by osteocytes. Consistent with this, sclerostin induces osteoblast apoptosis in vitro. Moreover, reduction of sclerostin levels by PTH and mechanical loading increases osteoblast number and activity as a result of stimulation of osteoblast differentiation and increased survival.[43,44] Activation of Wnt signaling in vitro by ligands known to activate the so-called canonical as well as noncanonical pathways also prevents apoptosis of osteoblast progenitors and differentiated osteoblasts through a mechanism that involves the Src/ERK and PI3/AKT pro-survival kinases.[45]

Glucocorticoids induce rapid bone loss resulting from a transient increase in resorption caused by delayed osteoclast apoptosis. This initial phase is followed by a sustained and profound reduction in bone formation and turnover caused by decreased osteoblast and osteoclast generation and increased osteoblast apoptosis.

Both persistent excess of PTH, as in hyperparathyroidism, and intermittent elevation of PTH (by daily injections) increase the number of osteoblasts. Sustained PTH elevation inhibits the expression of sclerostin, with a consequent increase in Wnt signaling and in differentiation of osteoblast precursors. A major effect of intermittent elevation of PTH is inhibition of apoptosis of osteoblasts, which thereby prolongs their life span and ability to form bone.

OSTEOCYTES

Osteocytes are former osteoblasts that become entombed during the process of bone deposition and are regularly distributed throughout the mineralized

bone matrix. Osteocyte bodies are individually encased in lacunae and exhibit cytoplasmic dendritic processes that run along narrow canaliculi excavated in the mineralized matrix. Osteocytes communicate with each other, with cells on the bone surface, and with cells of the bone marrow through gap junctions established between cytoplasmic processes of neighboring cells. Today it is accepted that osteocytes are the mechanosensory cells. Osteoblasts and osteoclasts are present on bone only transiently, in low number, and in variable locations. On the other hand, osteocytes are the most abundant resident cells and are present in the entire bone volume. Osteocytes are also the core of a functional syncytium that extends from the mineralized bone matrix to the bone surface and the bone marrow and all the way to the blood vessels. This strategic location permits the detection of variations in mechanical signals as well as in levels of circulating factors and allows amplification of the signals leading to adaptive responses.

Osteocyte apoptosis: consequences and regulation

Osteocytes are long-lived cells. However, similar to osteoblasts and osteoclasts, osteocytes die by apoptosis, and decreased osteocyte viability accompanies the bone fragility syndromes that characterize glucocorticoid excess, estrogen withdrawal, and mechanical disuse.[34] Conversely, preservation of osteocyte viability might explain at least part of the antifracture effects of bisphosphonates, which cannot be completely accounted for by changes in bone mineral density.[46]

Preservation of osteocyte viability by mechanical stimuli

Osteocytes interact with the ECM in the pericellular space through discrete sites in their membranes, which are enriched in integrins and vinculin, as well as through transverse elements that tether osteocytes to the canalicular wall. Loading of the bones induces ECM deformation and fluid flow through the canaliculi, producing tension in the tethering elements and strain on osteocyte membranes. The consequent integrin engagement leads to intracellular signaling. Physiologic levels of mechanical strain imparted by stretching or pulsatile fluid flow prevent apoptosis of cultured osteocytes. Mechanotransduction is accomplished by molecular complexes assembled at caveolin-rich domains of the plasma membrane and composed of integrins, cytoskeletal proteins, and kinases, including the focal adhesion kinase FAK and Src, which results in activation of the ERK pathway and osteocyte survival. Intriguingly, a ligand-independent function of the estrogen receptor is indispensable for mechanically induced ERK activation in both osteoblasts and osteocytes. This observation is consistent with reports that mice lacking estrogen receptor-α and estrogen receptor-β exhibit a poor osteogenic response to loading.

In vivo mechanical forces also regulate osteocyte life span. Apoptotic osteocytes are found in unloaded bones or in bones exposed to high levels of mechanical strain. In both cases, increased osteocyte apoptosis is observed before any evidence of increased osteoclast resorption. Apoptotic osteocytes accumulate in areas subsequently removed by osteoclasts. Targeted ablation of osteocytes in transgenic mice is sufficient to induce osteoclast recruitment and resorption, leading to bone loss. These findings led to the notion that dying osteocytes become the beacons for osteoclast recruitment to the vicinity and the resulting increase in bone resorption.[47] Whether living osteocytes continually produce molecules that restrain osteoclast recruitment or whether in the process of undergoing apoptosis osteocytes produce pro-osteoclastogenic signals remains to be determined. Taken together with the evidence that osteocyte apoptosis is inhibited by estrogens and bisphosphonates,[46,48] these findings raise the possibility that preservation of osteocyte viability contributes to the ability of these agents to inhibit remodeling.

Osteocyte apoptosis and aging

One of the purported functions of the osteocyte network is to detect microdamage and trigger its repair. During aging, there is an accumulation of microdamage and a decline in osteocyte density accompanied by decreased prevalence of osteocyte-occupied lacunae, an index of premature osteocyte death. Age-related loss of osteocytes caused by apoptosis could be partially responsible for the disparity between bone quantity and quality that occurs with aging. The decline in physical activity and thus reduced skeletal loading with old age is a potential mechanism for the increased prevalence of osteocyte (and osteoblast) apoptosis, as is the loss of estrogen in women during and after menopause.

Hormonal regulation of osteocyte life span

Estrogen and androgen deficiency both lead to increased prevalence of osteocyte apoptosis. Conversely, estrogens and androgens inhibit apoptosis of osteocytes as well as osteoblasts.[48] This antiapoptotic effect is due to rapid activation of the Src/Shc/ERK signaling pathway through nongenotropic actions of the classical receptors for sex steroids. This effect requires only the ligand-binding domain of the receptor, and unlike the classical genotropic action of the receptor protein, it is eliminated by nuclear targeting.

Excess of glucocorticoid activity in bone may also contribute to induction of osteocyte (and osteoblast) apoptosis because aged mice exhibit higher serum levels of corticosterone, elevated adrenal weight, and increased expression in bone of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), the enzyme that amplifies glucocorticoid action. The apoptotic effect of glucocorticoids is reproduced in cultured osteocytes and osteoblasts in a manner strictly dependent on the glucocorticoid receptor.[40] Induction of osteocyte and osteoblast apoptosis by glucocorticoids can result from the direct action of the steroids, because overexpression of the enzyme that inactivates glucocorticoids, 11β-HSD2, specifically in these cells abolishes the increase in apoptosis. Strikingly, in the osteocytic MLO-Y4 cell line, the proapoptotic effect of glucocorticoids is preceded by cell detachment caused by interference with FAK-mediated survival signaling generated by integrins. In this mechanism, Pyk2 (a member of the FAK family) becomes phosphorylated and subsequently activates proapoptotic JNK signaling. The proapoptotic actions of glucocorticoids may involve suppression of the synthesis of locally produced antiapoptotic factors, including IGF-1- and IL-6-type cytokines, as well as matrix metalloproteins, and stimulation of the proapoptotic Wnt antagonist SFRP-1.

Regulation of bone formation by osteocytes: sclerostin

Osteocytes express sclerostin, the product of the Sost gene, which antagonizes several members of the BMP family of proteins and also binds to LRP5/LRP6, preventing canonical Wnt signaling. Loss of Sost in humans causes the high-bone-mass disorders van Buchem syndrome and sclerosteosis. In addition, administration of an antisclerostin antibody increases bone formation and restores the bone lost after ovariectomy. Conversely, transgenic mice overexpressing Sost exhibit low bone mass.[49] These lines of evidence demonstrate that sclerostin derived from osteocytes exerts a negative feedback control at the earliest step of mesenchymal stem cell differentiation toward the osteoblast lineage. Moreover, PTH and mechanical loading downregulate the expression of sclerostin in osteocytes, which reveals a novel mechanism of bone anabolism triggered by osteocytes.

Osteocytes as mediators of the anabolic actions of canonical Wnt signaling in bone

Bone anabolic stimuli activate Wnt signaling, and human mutations of components along this pathway underscore its crucial role in bone accrual and maintenance. However, the cell responsible for orchestrating Wnt anabolic actions had remained elusive because genetic activation or deletion of components of the pathway in osteoblasts or their precursors only affect bone resorption without evident effects on bone formation.[50,51] A recent study demonstrates that activation of canonical Wnt signaling exclusively in osteocytes increases (not decreases) resorption and induces bone anabolism, leading to high bone remodeling with bone gain. This effect is due to the fact that activation of the pathway in osteocytes not only decreases OPG as in osteoblasts but also increases RANKL, leading to a higher RANKL-to-OPG ratio that favors resorption, and in this setting, Wnt signaling also favors osteoblast–osteocyte differentiation.[52] Thus, these findings demonstrate disparate outcomes of β-catenin activation in osteocytes versus osteoblasts and identify osteocytes as central target cells of the anabolic actions of canonical Wnt/β-catenin signaling in bone.

Regulation of bone resorption by osteocytes: RANKL and osteoprotegerin

The cues that signal bone resorption are not completely understood. Apoptotic osteocytes could regulate the recruitment of osteoclast precursors and their differentiation in two ways. Osteocyte apoptosis may indirectly stimulate osteoclastogenesis by inducing stromal/osteoblastic cells to secrete RANKL. In addition, osteocytes can directly secrete RANKL. Indeed, in vitro, purified osteocytes express higher levels of RANKL than osteoblasts and bone marrow stromal cells. The severe osteopetrotic phenotype observed in mice lacking RANKL in osteocytes and their resistance to bone loss induced by tail suspension strongly suggests that osteocytes are a major source of RANKL in vivo. Osteocytes also secrete OPG, which, as in osteoblasts, is regulated by the Wnt/β-catenin pathway. Mice lacking β-catenin in osteocytes are osteoporotic because of increased osteoclast numbers, but their osteoblast function is normal. Emerging evidence also points to osteocytes as an additional source of secreted M-CSF in bone. Together, these new findings suggest that osteocytes control the bone-remodeling process by regulating osteoclast and osteoblast differentiation and function.

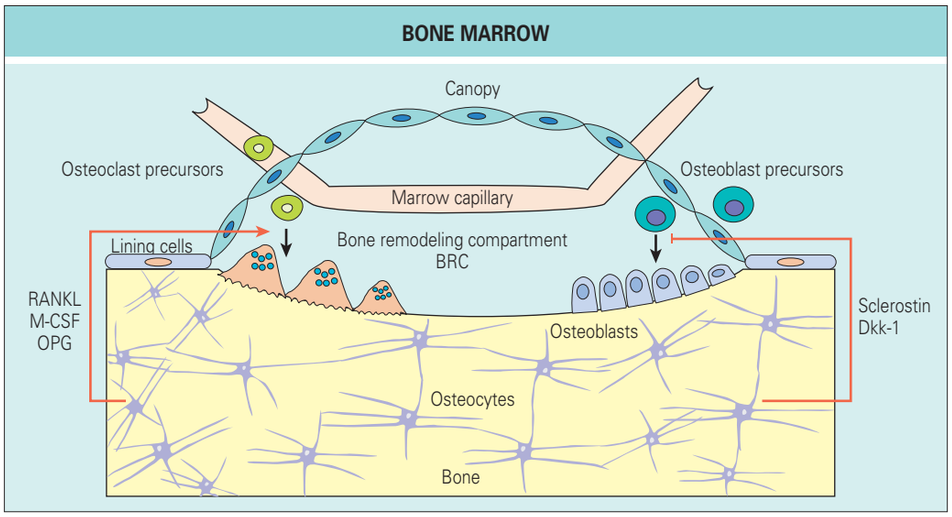

FIG. 3.7 Osteocytes sense the need for bone resorption and send signals to lining cells, which retract from the bone surface to allow the formation of a canopy under which remodeling occurs, called the *bone-remodeling compartment* (BRC). Osteoclast precursors are transported to the BRC by marrow capillaries, differentiate to mature osteoclasts under the influence of pro-osteoclastogenic and antiosteoclastogenic cytokines (receptor activator of nuclear factor-κB ligand [RANKL], macrophage colony-stimulating factor [M-CSF], and osteoprotegerin [OPG]) derived from osteocytes, and initiate bone remodeling. Osteoblast precursors from the bone marrow or the circulation differentiate into mature, bone-synthesizing cells in response to factors released from the bone matrix by resorption. Differentiation and function of osteoblasts are controlled by molecules derived from osteocytes, including sclerostin and Dkk-1. *(From Bellido T, Plotkin LI, Bruzzaniti A. Bone cells. In: Burr DB, Allen MR, editors. Basic and applied bone biology. San Diego: Academic Press; 2014, p. 27-46.)*

Table 3.1

Dynamic Processes of Skeletal Development and Adaptation

Process	Mechanism	Morphology	Function
Growth	F	Woven, lamellar	Increased mass
Modeling	A-F	Primary lamellar	Net increased mass *or* Adaption of architecture
	A-R	Control of drift and curvature	
Remodeling	A-R-F	Secondary lamellar (osteons, hemi-osteons)	Bone maintenance Repair of microdamage Prevention of bone loss
Repair	F	Woven Rapid mechanical adaptation	Repair of fractures

A, *Activation;* F, *formation;* R, *resorption.*

Osteocytes and the bone-remodeling compartment

Lining cells play an important function in initiating bone remodeling by retracting from quiescent bone surfaces and allowing the formation of a canopy over osteoclasts and osteoblasts in the bone multicellular unit.[53] On the endocortical surface, this canopy presumably encases bone marrow osteoblast precursors and is penetrated by blood vessels that provide hematopoietic osteoclast progenitors. The canopy, associated capillaries, osteocytes, osteoclasts, and osteoblasts form a compartment, the bone-remodeling compartment (BRC), which is separated from the rest of the marrow and which can sequester molecules that regulate the cells that remodel bone (Fig. 3.7). The signals that trigger lining cell detachment in a particular bone area are unknown. Premature apoptosis of osteocytes has been shown to precede osteoclast accumulation and resorption,[47] which raises the possibility that osteocytes release molecules that induce lining cell retraction facilitating access of osteoclast precursors to bone surfaces. However, the molecular entities responsible for this purported osteocytic function remain unknown. As discussed earlier, osteocytes express M-CSF,[54] which stimulates proliferation of preosteoclasts, and RANKL,[55,56] the master cytokine inducer of osteoclast differentiation, both of which could reach the BRC. Growth factors released from the bone matrix upon resorption, in turn, stimulate osteoblastogenesis. It is also likely that osteocyte-derived sclerostin reaching the BRC through the canalicular system and secretion of the other Wnt antagonist Dkk-1 by osteocytes as well as osteoblasts influence the rate of bone formation, providing an additional level of control of osteoblast activity. Based on these lines of evidence, the BRC might provide a supportive environment for differentiation of osteoclast and osteoblast progenitors. Thus, regulation of the bone-remodeling rate by hormonal and mechanical stimuli could be accomplished by controlling the balance between resorption and formation within the BRC through the regulation of osteocytic molecules, including sclerostin, RANKL, and OPG.

GROWTH, MODELING, REMODELING, AND REPAIR

Four dynamic processes are involved in skeletal development and adaptation. These are defined by the relationship of bone resorption and bone formation to each other (Table 3.1). These mechanisms include a coordinated system that first involves the activation (A) of cell populations followed by the resorption (R) of preexisting tissue and/or the formation (F) of new or replacement tissue. Bone growth serves to increase bone mass through bone formation. Resorption of bone is not part of the growth process, and the function of growth is only to increase mass, not to adapt the developing structure to its mechanical needs. Growth can occur on a substrate but may involve ossification directly from fibrous tissue (intramembranous bone formation) or by formation of a model with cartilage first and then replacement of the cartilage with bone (endochondral ossification). Modeling uses the tissue formed during the growth process to further increase bone mass and to shape its geometry to mechanical needs. Modeling occurs through the activation of cells followed by either formation or resorption. Formation and resorption are coordinated processes in modeling but do not occur sequentially on the same surface of bone. Remodeling, on the other hand, is defined by the sequential processes on the same bone surface of activation–resorption–formation (the A-R-F sequence). The function of remodeling is bone maintenance, not increase in bone mass, and the removal of microdamage. Bone's repair function, which restores its mechanical properties after a complete fracture or trabecular microfracture, usually occurs through the process of endochondral ossification, which forms a cartilage callus that also includes woven bone to bridge the fracture gap. This is eventually replaced through remodeling with replacement by lamellar bone.

Growth, modeling, and remodeling are present concurrently in all growing children. When skeletal maturity is reached, growth naturally stops. Modeling slows down or stops but may still be present at a reduced rate on trabecular

surfaces and on the periosteal surface of the bone. At maturity, the predominant process is bone remodeling, which maintains the bone that has been formed and repairs microscopic damage that may be sustained in bone during normal daily activities. Dysfunction in the remodeling process is associated with the loss of bone found in osteoporosis.

GROWTH

Growth of bone occurs through two different skeletal processes, one involving formation of bone from fibrous membranes and the other involving the formation of a skeletal anlage, or model. Intramembranous bone formation occurs at centers of ossification via direct mineralization in highly vascular fibrous tissues through the action of mesenchymal cells. The calvaria of the skull is the best example of intramembranous bone formation, with the individual bones of the skull acting as centers that eventually grow together at the sutures. Apposition of bone on the periosteal surface of long bones also occurs through intramembranous ossification.

In the long bones, development generally occurs by the initial condensation of mesenchyme or hyaline cartilage in the form of the eventual skeletal structure. This cartilage model mineralizes over time and becomes detectable as a primary center of ossification. Some bones are formed from a single ossification center, although most of the long bones form secondary centers of ossification at the ends (epiphyses), which eventually fuse to the bone that developed from the primary ossification center (diaphysis) (Fig. e3.2). The secondary centers allow growth to occur at a cartilaginous growth plate until skeletal maturity in the late teens or (for the vertebral bodies) in the early part of the third decade of life. The growth plate slowly converts into primary spongiosa that becomes remodeled into lamellar trabecular plates in the metaphysis of the bone.

MODELING

Long bones must grow both in length and in diameter (Fig. 3.8). At the ends of the long bones—near the epiphyses—growth of bone demands that the wider joint surface continually be reshaped and narrowed as it moves down into the metaphysis and diaphysis. Modeling is a continuous and prolonged process—unlike remodeling, which is episodic—and involves a coordinated process of bone resorption on some surfaces, while other surfaces undergo bone formation. Bone modeling occurs on both periosteal and endocortical envelopes and sculpts bone shape while allowing for expansion of the marrow cavity and periosteal diameter of the diaphysis. At the metaphysis, this occurs by osteoclastic resorption on the periosteal surfaces. As growth continues, however, bone is added to the periosteal surface by osteoblasts and simultaneously removed from the endocortical surface by osteoclasts. This increases whole-bone diameter and expands the marrow cavity, necessary for the formation of blood. It serves a second purpose: to increase the mechanical strength of the bone while at the same time not increasing its mass or weight at the same rate. Bone curvature is also adjusted during growth through a process known as *drift,* in which the periosteal surface on one side of the bone undergoes apposition while the opposite periosteal surface undergoes resorption. Likewise, different portions of the endocortical surface form or resorb bone in coordination to maintain the cortical thickness of the diaphysis.

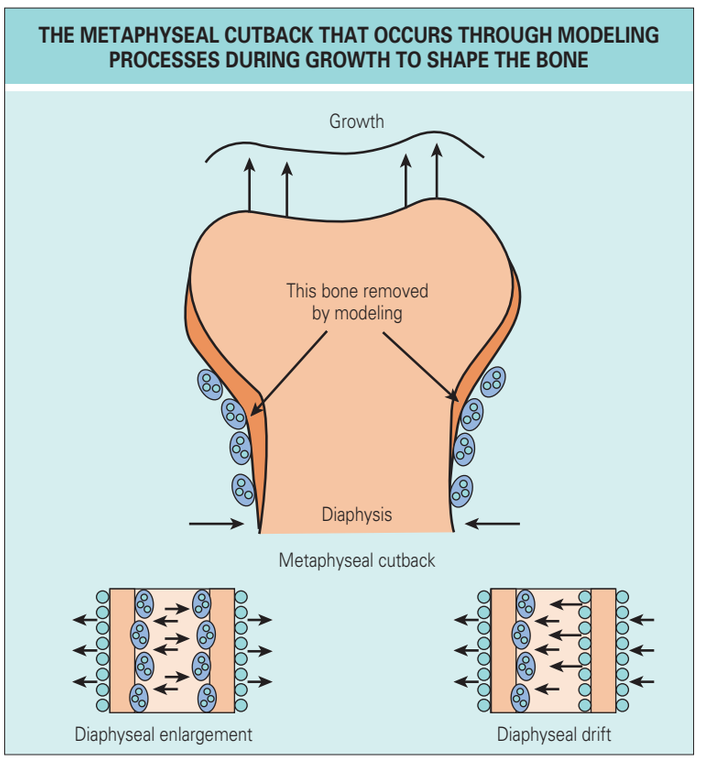

FIG. 3.8 During growth, metaphyseal bone is removed by modeling processes to shape the bone. Subsequent enlargement of the diaphysis occurs through direct periosteal apposition, which is often accompanied by resorption on the endocortical surface to enlarge the marrow cavity. Bone can also change its location and curvature through "drift." *Arrows* indicate the direction of drift, with *smaller circular cells* representing osteoblasts and bone formation and *larger ellipsoidal cells* representing osteoclasts and bone resorption. *(From Martin RB, Burr DB, Sharkey NA. Skeletal tissue mechanics. New York: Springer; 1998, p. 62, with permission.)*

REMODELING

The quantum concept of bone-remodeling states that bone is replaced in packets through the coupled activity of osteoclasts and osteoblasts. Coupling between osteoclasts and osteoblasts is the reason that it was difficult for so many years to control the processes involved in bone loss. When bone resorption is suppressed, formation is also suppressed because these activities are linked by intercellular signaling mechanisms that are not fully understood. In remodeling, resorption and formation are coupled but in fact may not be balanced. Coupling and balance are not the same; whereas *balance* refers to the relationship between the amount of bone resorbed and the amount formed, *coupling* denotes only that the processes are linked in some way. Resorption and formation are in balance in the healthy skeleton, but when these are out of balance, the amount of bone that is resorbed can be either greater or less than the amount that is subsequently formed. Thus, even though cells are coupled, bone can be lost or added in several different ways based on the altered balance of resorption and formation. In actuality, one almost never finds a balance in favor of bone formation in a remodeling system, although this has been shown to occur with anabolic treatments for osteoporosis, such as the intermittent administration of recombinant human PTH(1-34) (Teriparatide).[57] More often, the balance is in favor of resorption. This is the case in osteoporosis, in which global resorption is increased but formation at each of the erosion sites is normal or reduced, which leads to a deficit in bone mass.

This coupled system is termed a *bone multicellular unit* (BMU) because different cell populations are involved. A BMU typically consists of about 10 osteoclasts and several hundred osteoblasts. When cut in longitudinal section, the BMU shows the sequential aspects of the A-R-F system and the various cell populations that are involved (Fig. e3.3). Each of the phases in this sequence is location, magnitude, and rate specific, so that alterations in the magnitude or timing of one can produce morphologic features characteristic of specific skeletal abnormalities (Fig. 3.9). Activation is initiated by chemical or mechanical signals but actually involves a series of events that include recruitment of precursor cells, differentiation and proliferation of cells, and migration to the site of activity. In humans, these processes take about 5 to 10 days. Bone resorption by mature osteoclasts takes about 3 weeks at a given site, although osteoclasts moving longitudinally through bone at a rate of about 40 μm/day may live much longer than this. There is a period of reversal during which there is neither bone resorption nor formation; this may represent a period like the activation period during which osteoblasts are undergoing differentiation and proliferation from their precursors. This is followed by a period of bone formation that lasts about 3 months. As unmineralized bone—or osteoid—is laid down, it subsequently begins to mineralize, quickly at first, and then more slowly over the following year. This sequence of events occurs on all four skeletal envelopes (periosteal, endocortical, trabecular, and intracortical).

Changes in bone mass can occur simply through changes in activation frequency. Changes in activation frequency may lead only to transient changes in bone mass if resorption and formation are in balance. Early losses of bone mass solely caused by increased activation frequency may resolve after several months as the newly resorbed sites are refilled. Likewise, bone mass may change because some aspect of the recruitment, proliferation, migration, or differentiation of either osteoclasts or osteoblasts is interrupted. These changes may be manifested as alterations of resorption–formation balance but may be caused by activation defects during cell maturation.