VOLUME 1

The Cytokine Handbook

FOURTH EDITION

EDITED BY
Angus W Thomson
Michael T Lotze

GH PRO EPO IL2 IL3 IL4 IL5 IL6 IL7 IL9 IL11 IL12
IL23 IL12RA IL27 IL13 IL15 IL21 IL16 IL17 IL25 GM
GCSF IFNα IFNβ IFNω IL28A
IL28B IL29 IFNγ IL10 IL19 IL20
IL22 IL24 IL26 IL1F IL1F10
IL1RA IL18 FGF HGF TLR
LTα LTβ TNF TRAIL RANK L
FasL Fas HMGB1 MCSF
EGF Flt3L SCF VEGF MIF IL8 CXC C-C TGFβ BMP

The Cytokine Handbook

Fourth edition

VOLUME I

This Page Intentionally Left Blank

The Cytokine Handbook

Fourth edition

VOLUME I

Edited by

Angus W. Thomson
Starzl Transplantation Institute
University of Pittsburgh School of Medicine, USA

Michael T. Lotze
Molecular Medicine Institute
University of Pittsburgh School of Medicine, USA

ACADEMIC PRESS

An imprint of Elsevier Science

Amsterdam • Boston • London • New York • Oxford • Paris
San Diego • San Francisco • Singapore • Sydney • Tokyo

This book is printed on acid-free paper

First edition published 1991
Second edition published 1994
Third edition published 1998
Fourth edition published 2003

Academic Press
An Imprint of Elsevier Science
84 Theobald's Road, London WC1X 8RR, UK
http://www.academicpress.com

Academic Press
An Imprint of Elsevier Science
525 B Street, Suite 1900 San Diego, California 92101–4495, USA
http://www.academicpress.com

ISBN 1–12–689663–1

Library of Congress Catalog Number: 2002114101

British Library cataloguing in Public Data
Vol.I
1. Cytokines – Handbooks, manuals, etc. 2. Chemokines – Handbooks, manuals, etc.
I. Thomson, Angus W. II. Lotze, Michael T.
611'.0185

Typeset by J&L Composition, Filey, North Yorkshire
Printed and bound in Great Britain by The Bath Press, Avon

Contents

Volume 1

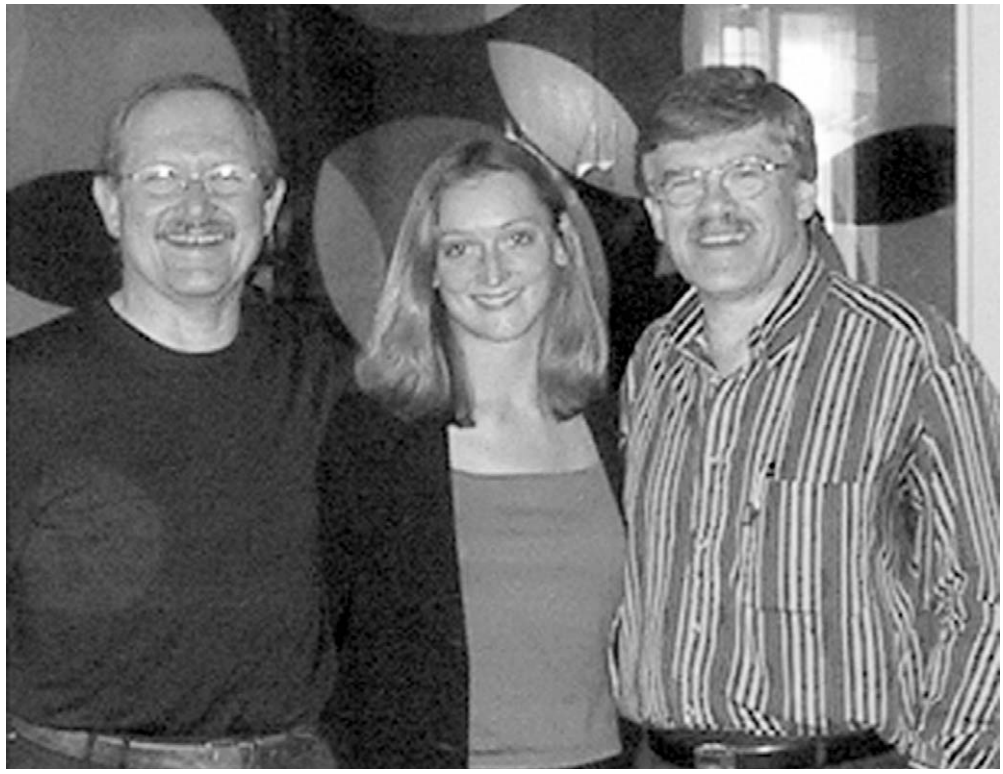
Volume II

Dedications

For Robyn, Andrew, Natalie and Emma
Angus Thomson

For Joan, Thomas, Anna, Mac and Jenny
Michael Lotze

This Page Intentionally Left Blank



Editorial Meeting, Stockholm/International Immunology Congress, July 2001. Drs. Lotze (left), Read (center), and Thomson (right).

Preface to the First Edition

Cytokines feature at the forefront of biomedical research. An understanding of their properties is now essential for the immunology student, researcher and teacher and for today's medical practitioner who needs to understand immunologic disease and immunological approaches to therapy. The pace with which this ever-expanding field has developed has been rapid enough to exceed the most optimistic expectations and to bewilder the most assiduous student. Cytokine research is expected to provide the key to pharmacological manipulation of the immune response and commands the attention of a massive and highly focused biotechnology industry. The chapters in this book are a good representation of the areas to which molecular biology has been most successfully applied. Biotechnology companies provide most of the pure, well-characterized cell growth regulatory and effector molecules used in academic and industrial laboratories or in clinical medicine as diagnostic tools or therapeutic agents.

Cytokines represent a sought-after symposium theme in immunology, molecular biology and molecular genetics. The cytokine literature ranges from the most basic to the applied. Unfortunately, technical advances, rapid expansion and diversification have prompted narrower specialization and reduced the ease of communication. The aim of this book is to inform and to provide detailed information and reference material on the many aspects of pure and applied cytokine science. These include the molecular characteristics of cytokines, their genes and receptors, the cellular sources and targets of cytokines, their biological activities and as best can presently be defined, their mechanisms of action. Confronted with such a vast amount of new information, up-to-date coverage is an almost unattainable goal. The scope of cytokine research could only be effectively covered in a multi-authored volume and it is indeed fortunate that each chapter is written by a leading authority(ies). Although many chapters focus on individual cytokines, it is also apparent that aspects such as cell sources, molecular structure, purification and bioassay have many features in common. A certain amount of duplication is, therefore, inevitable. The cytokine network, cytokine interactions, the roles of cytokines in disease pathogenesis and the therapeutic applications of cytokine research are dealt with in detail. In attempting to provide comprehensive coverage, a chapter on phylogeny has been included. The last chapter was commissioned to provide both perspective and a somewhat sobering view of the future.

I am indebted to the many authors around the world who have so generously devoted their knowledge, energy and time to the creation of this book. I also wish to acknowledge the support of Dr Susan King, and her staff at Academic Press in London, whose skill and energy were essential in the genesis of *The Cytokine Handbook*.

Angus W. Thomson
University of Pittsburgh

This Page Intentionally Left Blank

This Page Intentionally Left Blank

Preface to the Second Edition

In the Preface to the First Edition, it was stated that to produce a book that provided up-to-date coverage of all aspects of the cytokine field was an 'almost unattainable goal'. Since the First Edition went to press in the spring of 1991, advancement both in our knowledge and understanding of the cytokine network has been predictably rapid, fully justifying the publishers' faith in a Second Edition of *The Cytokine Handbook* within 3 years. The original chapters have been revised and updated, and, with few exceptions, this has been undertaken by the original authors. Synthesis of information in the context of the cytokine network has again been a key objective. Every effort has been made to maintain currency and it is in promptly fulfilling this goal that the contributing authors deserve great credit. 'New' cytokines have, of course, emerged and continue to 'appear'. Thus, this new edition features individual chapters on IL-9, IL-10 and IL-12, with a 'stop-press' overview of IL-13 and even newer molecules that are candidates for designation as interleukins IL-14 and IL-15.

What constitutes a new interleukin? The assignation of a new designation depends on several clearly defined criteria, recently established by a sub-committee of the nomenclature committee of the International Union of Immunological Societies (Paul *et al.*, 1992). The criteria laid down include molecular cloning and expression, a unique nucleotide and inferred amino acid sequence, and the availability of a neutralizing monoclonal antibody. Furthermore, the granting of a new interleukin designation requires that the candidate molecule be a natural product of cells of the immune system (defined loosely as lymphocytes, monocytes and other leukocytes). The new interleukin must also mediate a potentially important function in immune responses and exhibit an additional function(s) so that a simple, functional name might not be adequate. Finally, these characteristic features should have been described in a peer-reviewed publication.

This new edition incorporates separate chapters on G-CSF, M-CSF and GM-CSF, whereas in the first edition, only one chapter – on 'colony stimulating factors' – covered the properties of these molecules. A chapter is also now afforded to *TGF-β*, which exhibits both inhibitory and stimulatory effects on a variety of cell types and is a potent immunosuppressant. Another significant and substantial new chapter concerns 'chemokines'. IL-8 (the 'highest' interleukin designation afforded a separate chapter in the first edition) was the first member of the chemokine family to be identified. More recently, other low molecular weight chemotactic polypeptides have been discovered that play a key role in cell activation and chemotaxis during the inflammatory response. These include RANTES (regulated upon activation, normal *T* expressed and secreted!), macrophage chemotactic and activating factor (MCAF), macrophage inflammatory protein (MIP)-l*α* and MIP-1*β*. All share a conserved, four cysteine motif and can be further subclassified on the spacing of the conserved cysteine residues.

Evaluation of the clinical potential of cytokines is one of the most exciting challenges of contemporary medicine. In addition, and in contradistinction to cytokine or cytokine gene therapy, the emergence of a new class of therapeutic agents, comprising soluble cytokine receptors (e.g. soluble (s) IL-IR, sTNFR, sIFN-γR), receptor antagonists (e.g. IL-1ra) and counter-regulatory cytokines, represents one of the most important developments in the cytokine field in recent years. Successes in the sequencing, cloning and expression of cytokine receptors has facilitated production and evaluation of their therapeutic utility in several acute and chronic cytokine-mediated diseases. This exciting and challenging development is one of the themes covered under the several chapters devoted to therapeutic aspects of cytokine biology.

The last few years have seen the advent of new approaches to interrogating the roles of cytokines *in vivo*. Thus cytokine gene 'knockout' mice and mice transgenic for cytokine gene reporter constructs are likely to provide new knowledge about the *in vivo* role(s) of cytokines, especially in experimental autoimmune disorders, cancer or infectious diseases that may be difficult to mimic satisfactorily *in vivo*. The molecular genetics of cytokines (reviewed in Chapter 2) is yet another new and exciting aspect of this key field of contemporary molecular biology and medicine that has provided the impetus for the second edition of *The Cytokine Handbook*. Those who acquire knowledge by reading this book, or who are stimulated by the implications of the recent developments described herein, owe thanks to the many experts around the world who have so generously given of their valuable time, energy and expertise. The cordial and enthusiastic support of these scientists and clinicians has been an impelling influence that would be difficult to overrate. I am indebted to my colleague Dr Mike Lotze for constructive suggestions, Ms Shelly Conklin for valuable secretarial help and to Dr Tessa Picknett and her colleagues at Academic Press in London for their resolute support and guidance in ensuring that the notion of a second edition became a tangible reality.

Angus W. Thomson

REFERENCE

Paul, W.E., Kishimoto, T., Melchers, F. *et al.* (1992). *Clin. Exp. Immunol.* **88:** 367.

Preface to the Third Edition

As the Third Edition goes to press, the stream of cytokine discovery flows unabated. We are confronted by description after description of novel cytokine-controlled systems of cell differentiation and proliferation and of the role of cytokines in immune regulation. In order to keep pace with these developments, this new edition of *The Cytokine Handbook* has been completely revised and updated. The contributors are largely the same body of devoted international authorities, with several notable new additions. Amongst the newcomers to the extensive panoply of cytokines covered in this edition are the recently designated interleukins (IL)-16 (formerly lymphocyte chemotactic factor), IL-17 (identified originally as cytotoxic T lymphocyte antigen-8) and IL-18 (interferon-γ inducing factor). The daunting expansion of new information is perhaps better illustrated by the 'explosion' of chemokines. About sixty distinct human chemokine gene products have been identified to date. Regarded historically as regulators of leukocyte trafficking, chemokines have recently made a major impact in the area of infectious disease, including dramatic new understanding that chemokine receptors are at the center of HIV pathogenesis. Just two examples of chemokines that have recently come to prominence are eotaxin (the CCR-3 receptor-specific, eosinophil-selective chemokine) and the structurally unique fractalkine, with intrinsic adhesion and chemotactic activities. Two chapters are now devoted to the vastly expanding area of chemokines and their receptors.

The Third Edition sees individual chapters afforded to IL-1 through IL-15, with the exception of IL-14, reflecting the current uncertainty about the identity of the IL-14 molecule. The TNF-related cytokine-receptor superfamily of secreted and membrane-bound ligands that includes TNF/lymphotoxin (α/β, CD40, Fas and 4–1BB systems, now appears to have important functional roles in the immune response. Each member is paired with a specific cell surface receptor(s) that, together, form a corresponding family of receptors. Recent studies have revealed important and unique roles of the TNF- related ligands and receptors that are covered in an additional new chapter.

In addition to coverage of the colony-stimulating factors G-, M- and GM-CSF, two new chapters in this edition are afforded to individual hematopoietic cytokines – stem cell factor (SCF) (c-kit ligand) and flt-3 ligand – that have many biological functions in common. SCF is a potent costimulatory or synergistic factor in cytokine cocktails for manipulation of hematopoietic cells. It appears to be of value in the combination of *ex vivo* 'expansion' technologies with gene transfer methods for the correction of genetic disease or the support of multiple chemotherapy cancer patients. The recently cloned cytokine flt-3 ligand has been shown to dramatically increase the numbers of functional dendritic cells and also to increase natural killer cells *in vivo*. Moreover it exerts anti-tumor activity, raising expectation of a future role of this molecule as a human immunotherapeutic agent.

Therapeutic applications of cytokines are now covered in detail in several chapters, including a new chapter on cytokine gene therapy. The first therapeutic cytokine gene transfer study began several years ago in patients with advanced malignancy; now numerous trials have been approved for the transfer of cytokine genes to tumor cells, tumor-infiltrating lymphocytes, blood lymphocytes, or fibroblasts. A few studies involve the use of cytokine genes for the treatment of non-malignant conditions, such as rheumatoid arthritis. A new chapter in this edition reviews progress in cytokine gene therapy.

As in the two previous editions, valuable up-to-date practical information is included in the extensive account of cytokine assays. The phylogeny of cytokines has become such a growth area that the information can barely be contained in a single chapter.

I am again immensely indebted to the many experts around the world who have made this new edition possible. On the home front, my colleague Dr Mike Lotze has been a constant source of creative suggestions and lively discussion. My thanks are due once again to Dr Tessa Picknett, senior editor, and also to Mr Duncan Fatz and Ms Emma White at Academic Press in London, and to Ms Shelly Conklin for invaluable secretarial assistance.

Angus W. Thomson

Preface to the Fourth Edition

> It is also said that Sisyphus, being near to death, rashly wanted to test his wife's love. He ordered her to cast his unburied body into the middle of the public square. Sisyphus woke up in the underworld. And there, annoyed by an obedience so contrary to human love, he obtained from Pluto permission to return to earth in order to chastise his wife. But when he had seen again the face of this world, enjoyed water and sun, warm stones and the sea, he no longer wanted to go back to the infernal darkness. Recalls, signs of anger, warnings were of no avail. Many years more he lived facing the curve of the gulf, the sparkling sea, and the smiles of earth. A decree of the gods was necessary. Mercury came and seized the impudent man by the collar and, snatching him from his joys, led him forcibly back to the underworld, where his rock was ready for him.
>
> **The Myth of Sisyphus, Albert Camus; 1940.**

Camus was awarded the Nobel Prize for Literature in 1957 for his existentialist contributions to finding order and meaning for man living in a world absurd and, at the time he wrote it, wracked by war. The notion of a mortal committed to daily striving to move his rock uphill serves as a suitable metaphor for cytokines, literally cell movers. At some level it also mirrors the profound labors of our contributors who, in each edition, find the energy to move their chapters a bit closer to the apogee of fuller understanding and bounded exposition. If this work is at all successful, it is because of them and their professorial stance to organizing what is becoming almost an impossibly large set of contributions to the literature.

Astute observers will find this fourth edition substantially altered, now in two volumes, organized around cytokine families, many of them revealed by the availability of new sequence data arising from the human genome project. It also introduces quotes chosen in most part by the author of each chapter and denoting some of the personal affectations of each contributor. It has striven to be complete and still readable allowing access to the considerable literature now available for many of these factors which, Mercury-like, bring and move cells to their appointed fate. The final section knits together the individual cytokines into the clinical implications and, in some instances, applications of cytokines. The hope is that some of them might find their way singly, or most often, in pairs, into useful recombinant pharmaceutical agents. Since the last edition, interleukin 1 receptor antagonist (IL-1RA) has

been approved for the therapy of rheumatoid arthritis (Schiff, 2000). The successful application of TNF antagonists in chronic inflammatory conditions continues to expand with what we suspect will be an increasing number of indications moving beyond arthritis (Criscione and St Clair, 2002) and inflammatory bowel disease to other difficult clinical problems. Up to the moment of press, we have been cognizant of new molecules and new insights and have incorporated these with assistance and support of our editor up to the recently identified IL-28 and IL-29! (Kotenko *et al.*, 2002; Sheppard *et al.*, 2002).

Some of the major advances captured in this edition beyond those made possible by the availability of the full human and mouse genome represent the increased power of new technology. These include application of quantitative polymerase chain reaction (PCR) assessment of cytokines (Walker, 1998; Gulietti *et al.*, 2001; Rajeevan *et al.*, 2001), availability of measurement of multiple cytokines in ELISpot assays (Bennouna *et al.*, 2002; Leong *et al.*, 2002; Meidenbauer *et al.*, 2002; Scheibenbogen *et al.*, 2002; Shmitz *et al.*, 2002) as well as microarrays (Benson *et al.*, 2002; Cappellen *et al.*, 2002) allowing careful evaluation of the downstream messages induced by cytokines and chemokines. The proteomic analysis and patterning of individual cytokines *in vitro* and *in vivo* is less fully developed (Petricoin *et al.*, 2002; Schweitzer *et al.*, 2002) but by the time of the fifth edition is expected to allow greater analysis of the complexity of cell communication mediated by cytokines. Also the deeper understanding of individual cells or cell mixtures has been made possible by the development of so-called high content screening using integrated high resolution fluorescence microscopy, data algorithm development and display and automation (Ghosh *et al.*, 2000; Taylor *et al.*, 2001). We anticipate that studying cells interacting with each other will provide a wealth of additional information, as yet only modestly pursued in the available literature.

We would like to dedicate this edition of *The Cytokine Handbook* to our colleagues, the scientists laboring within academe and the biotechnology/pharmaceutical industries. Like Sisyphus and his spouse, they have to work long hours, often together, to move their chosen cytokine or chemokine closer to a fuller understanding and ultimate utility in discerning the biology writ large by their production, in full display within the public square. It is our expectation that within the bounds of creating new knowledge and creating value, that application of this knowledge might improve human health and the common weal. Finally to Shelly Conklin who organized our manuscripts dutifully as they each arrived, to Bridget Colvin for her scholarly contribution to the quotes, and to Jacqueline Read who together with Tessa Picknett as our editors, made the task of developing this edition more joyful than if it were in less able hands, we offer our humble appreciation and thanks.

Michael T. Lotze
Angus W. Thomson

REFERENCES

Bennouna, J., Hildesheim, A., Chikamatsu, K. *et al.* (2002) Application of IL-5 ELISPOT assays to quantification of antigen-specific T helper responses. *J. Immunol. Method*s **261**(1–2): 145–156.

Benson, M., Svensson, P.A., Carlsson, B. *et al.* (2002) DNA microarrays to study gene expression in allergic airways. *Clin. Exp. Allergy* **32**(2): 301–308.

Cappellen, D., Luong-Nguyen, N.H., Bongiovanni, S. *et al.* (2002) Transcriptional program of mouse osteoclast differentiation governed by the macrophage colony-stimulating factor and the ligand for the receptor activator of NFkB. *J. Biol. Chem.* **277**: 21971–21982.

Criscione, L.G. and St Clair, E.W. (2002) Tumor necrosis factor-alpha antagonists for the treatment of rheumatic diseases. *Curr. Opin. Rheumatol.* **14**(3): 204–211.

Ghosh, R.N., Chen, Y.T., DeBiasio, R. *et al.* (2000) Cell-based, high-content screen for receptor internalization, recycling and intracellular trafficking. *Biotechniques* **29**(1): 170–175.

Gulietti, A., Overbergh, L., Valckx, D. *et al.* (2001) An overview of real-time quantitative PCR: applications to quantify cytokine gene expression. *Methods* **25**(4): 386–401.
Kotenko, S.V., Gallagher, G., Baurin, V.V. *et al.* (203) IFN-λ mediate antiviral protection through a distinct class II cytokine receptor complex. *Nature Immunology* **4**, 69–77.
Leong, S.P., Peng, M., Zhou, Y.M. *et al.* (2002) Cytokine profiles of sentinel lymph nodes draining the primary melanoma. *Ann. Surg. Oncol.* **9**(1): 82–87.
Meidenbauer, N., Gooding, W., Spitler, L. *et al.* (2002) Recovery of zeta-chain expression and changes in spontaneous IL-10 production after PSA-based vaccines in patients with prostate cancer. *Br. J. Cancer* **86**(2): 168–178.
Petricoin, E.F., Ardekani, A.M., Hitt, B.A. *et al.* (2002) Use of proteomic patterns in serum to identify ovarian cancer. *Lancet* **359**(9306): 572–577.
Rajeevan, M.S., Ranamukhaarachchi, D.G., Vernon, S.D. and Unger, E.R. (2001) Use of real-time quantitative PCR to validate the results of cDNA array and differential display PCR technologies. *Methods* **25**(4): 443–451.
Scheibenbogen, C., Sun, Y., Keilholz, U. *et al.* (2002) Identification of known and novel immunogenic T-cell epitopes from tumor antigens recognized by peripheral blood T cells from patients responding to IL-2-based treatment. *Int. J. Cancer* **98**(3): 409–414.
Schiff, M.H. (2000) Role of interleukin 1 and interleukin 1 receptor antagonist in the mediation of rheumatoid arthritis. *Ann. Rheum. Dis.* **59**(Suppl 1): i103–i108.
Schweitzer, B., Roberts, S., Grimwade, B. *et al.* (2002) Multiplexed protein profiling on microarrays by rolling-circle amplification. *Nat. Biotechnol.* **20**(4): 359–365.
Sheppard, P., Kindsvogel, W., Xu, W. *et al.* (2003) IL-28, IL-29 and their class II cytokine receptor IL-29R. *Nature Immunology* **4**, 63–68.
Shmitz, M., Rohayem, J., Paul, R. *et al.* (2002) Quantification of antigen-reactive T cells by a modified ELISPOT assay based on freshly isolated blood dendritic cells. *J. Clin. Lab. Anal.* **16**(1): 30–36.
Taylor, D.L., Woo, E.S. and Giuliano, K.A. (2001) Real-time molecular and cellular analysis: the new frontier of drug discovery. *Curr. Opin. Biotechnol.* **12**(1): 75–81.
Walker, K.B. (1998) Detection and analysis of cytokine mRNA in tissues and cell lines. *J. Immunol. Methods* **212**(1): 113–123.

This Page Intentionally Left Blank

Foreword to the Fourth Edition

What are cytokines?
As so aptly discussed by Jan Vilcek in the introductory chapter to this book, The Cytokine Handbook, cytokines are regulatory peptides that can be produced by every nucleated cell type in the body. Cytokines have pleiotropic regulatory effects on haematopoietic and many other cell types that participate in host defense and repair processes. Cytokines therefore include lymphocyte-derived factors known as 'lymphokines', monocyte-derived factors called 'monokines', haematopoietic 'colony stimulating factors', connective tissue 'growth factors', and chemotactic chemokines.

Why do cytokines exist?
The evolution of large multicellular organisms require the development of intercellular messengers such as hormones, neuropeptides and cytokines to permit marshalling of co-ordinated cellular responses. It has been proposed that the structural homology between adhesion proteins that mediate cell-contact-dependent interactions and cytokine ligands, suggests that soluble cytokines evolved from cell-associated signals (Grumet *et al.*, 1991). In fact, a number of cytokines and their receptors exist in both soluble (shed) and cell-associated form and can have different functional consequences in each state.

How are cytokines different from hormones?
Endocrine hormones, which are generally produced by specialized glands, are present in the circulation and serve to maintain homeostasis. In contrast, most cytokines usually act over short distances as autocrine or paracrine intercellular signals in local tissues and – with the exception of macrophage colony-stimulating factor (M-CSF), stem cell factor, erythropoietin and a latent form of the transforming growth factor β (TGFβ) – only occasionally spill over into the circulation and initiate systemic reactions. Except for the above, cytokines generally are not produced constitutively, but are generated in response to danger signals to contend with challenges to the integrity of the host. The functions of cytokines are distinct from those of hormones, since they serve to maintain homeostasis by regulating innate host defense and the immune system, through damage control and by promotion of reparative processes.

How did lymphokines come to be discovered?
The possibility that cell-derived factors mediate biological activities was first suggested by experiments carried out by Rich and Lewis (1932). They observed that migration of neutrophils and macrophages in

cultures of tuberculin-sensitized tissues was inhibited by antigen. Waksman and Matoltsy (1958) observed that macrophages in monolayer cultures were actually stimulated rather than damaged by exposure to tuberculin antigens. George and Vaughan (1962) improved the technique of evaluating migration of mononuclear cells using capillary tubes. The study of 'lymphokines' was initiated concurrently by David *et al.* (1964) and Bloom and Bennet (1966). They used this technique to show that antigens could stimulate sensitized lymphocytes in cultures to produce macrophage migration inhibitory factors (MIF). At about the same time, supernatants of mixed leukocyte cultures were found by Kasakura and Lowenstein (1965) to be 'blastogenic' for lymphocytes. This 'blastogenic factor' (BF) was subsequently called 'lymphocyte mitogenic factor' (LMF). This was followed by the discovery of a variety of lymphocyte-derived biological activities in culture supernatants. Ruddle and Waksman as well as Kolb and Granger described a cytotoxic lymphocyte-derived mediator called 'lymphotoxin' (LT) in 1968.

In vitro monocyte migration in response to supernatants of antigen activated lymphocytes was attributed to a lymphocyte-derived chemotactic factor (LCF) (Ward *et al.*, 1969) and was correlated with the recruitment of mononuclear cells to *in vivo* inflammatory sites. MIF and the macrophage aggregation factor (MAgF) (Lolekha *et al.*, 1970) presumably served to retain cells at inflammatory sites. The necrotic centers of some granulomas could be attributed to the cytodestructive activity of LT (Kolb and Granger, 1968; Ruddle and Waksman, 1968), and the presence of lymphoblasts and frequent mitotic figures was mediated by LMF (Kasakura and Lowenstein, 1965). Moreover, identification of macrophage-activating factor (MAF) (Nathan *et al.*, 1971) and lymphocyte-derived immune interferon (IFN-γ) (Green *et al.*, 1969) provided a biological basis for acquired resistance to infectious organisms. Consequently, the various biological activities secreted by cultured antigen-stimulated lymphocytes provided *in vitro* models for the pathogenesis of *in vivo* delayed hypersensitivity reactions. These biochemically undefined, lymphocyte-derived activities were termed 'lymphokines' in 1969 by Dumonde *et al.* Discovery of the lymphokines revolutionized the conceptual basis of cell-mediated immunity and these biological activities were considered '*in vitro* correlates' of cell-mediated immunity.

What led to the recognition that lymphokines were members of the cytokine family?
In 1971–1974 Gery and coworkers showed that the lymphocyte-activating factor (LAF) was produced by adherent monocytes and macrophages (Gershon and Kondo, 1971; Gery *et al.*, 1971; Gershon *et al.*, 1974). This was the first demonstration of the existence of non-lymphocyte-derived 'monokines'. Based on this information and on his own observations that some replicating non-lymphoid cell lines, as well as virally infected non-lymphoid cells, could also produce lymphokine-like MIF and chemotactic factors, Cohen proposed that all these mediators including lymphokines should therefore be called 'cytokines' (Cohen *et al.*, 1974). This resulted in the conceptual transformation of lymphokines from subjects of interest to a minor subset of immunologists, to cytokines that function as bidirectional intercellular signals between somatic and myeloid as well as lymphoid cells, with potential impact on a great number of biological disciplines.

What developments galvanized the study of cytokines?
The development of tissue culture techniques in the 1960s enabled immunologists to detect the presence of factors in tissue culture supernatants and enabled them to perform *in vitro* studies of the mobility, proliferation, differentiation and functional capabilities of lymphocytes and other leukocytes. Cytokines are very potent and active at pM to nM concentrations. Thus, they are active at only trace levels. This makes it particularly difficult to isolate and identify the biochemical structure of these peptides. These factors, which were originally disparagingly termed 'lymphodrek', could be purified only with the development of improved chromatography and microsequencing techniques in the late 1970s. The fortuitous development of molecular biology and monoclonal antibody technologies accel-

erated the identification of cytokines in the 1980s ensuring the availability of abundant quantities of recombinant cytokines over the past decade. This has resulted in an information explosion that is reflected by *The Cytokine Handbook*.

What was the origin of the interleukin terminology?
By 1978, the confusing plethora of eponyms in existence for monocyte and lymphocyte-derived activities motivated investigators at the Second International Lymphokine Workshop – held near Interlaken, Switzerland – to propose more inclusive 'neutral' terms for these biological activities. The researchers recognized that the numerous monokine and lymphokine activities which they were detecting, along with a variety of bioassays, actually had numerous properties in common. This resulted in the erroneous impression that these cytokine activities, each with their own name, could all be attributable to one or two molecular entities. Paetkau proposed that monocyte-derived LAF/BAF/MCF be renamed interleukin-1 (IL-1), while lymphocyte-derived LMF/BF/TCGF should be called IL-2 (Mizel and Farrar, 1979). The interleukin terminology symbolized the broader roles of these cytokines and, with the progressive increase in the number of interleukins, now up to 27, has led to an explosive increase in the interest of investigators from a variety of disciplines in these mediators of inflammation and immunity.

Why is the cytokine nomenclature so chaotic?
Of the more than 200 cytokines that have been cloned to date, many retain their initial names, which usually denote only the functional activity that led to their discovery. Chaotic as this may be, such names are easier to recall than an interminable number of interleukins or chemokines. A minority of investigators request an interleukin designation to focus greater attention on their discovery. To qualify as an interleukin, the cytokine must be documented to have a unique amino acid sequence and functional activity involving leukocytes. The evidence is evaluated by the nomenclature and standardization committee of the International Cytokine Society and the Union of Immunological Societies who make a recommendation to the World Health Organization. Despite this rather straightforward process, committees have had to resolve conflicts concerning simultaneous claims to the IL-4 number and even a fraudulent claim (e.g. IL-4a). More recently, an error in the nucleotide sequence of the precursor region of IL-16 led to a re-evaluation of the IL-16 designation. Fortunately, the error did not involve the region of the gene coding for the mature protein and the translated amino acid sequence proved to be correct and exhibited the proposed functions of IL-16. In contrast, the nucleotide and consequent amino acid sequence of IL-14 have proven to be incorrect. Unfortunately, the correct protein sequence of IL-14, if any, remains unknown. It is therefore unclear whether IL-14 exists; hence its omission from this Handbook. Perhaps such errors can be prevented by requiring that novel cytokine sequences be independently confirmed to be eligible for an interleukin designation. Structurally distinct interleukins that use the same receptor have been given a number followed by a Greek letter, as for example IL-1α and IL-1β.

Is there any order to the cytokine chaos?
Despite the identification of many structurally distinct cytokines, they can be organized into groups that exhibit functional similarities based on shared receptor utilization. This holds for IL-1α and IL-1β as well as TNFα and LT which use shared and unshared receptors. Receptor chains are shared by a number of the cytokine groups. The IL-2γ receptor chain is shared by IL-2, 4, 7, 9, 15 and 21. The gp130 chain of the IL-6 receptor is shared by IL-6, IL-11, LIF, oncostatin-M, CNTF and cardiotrophin-1. Members of the TNF family of ligands share receptors with homology to the TNF receptors. This includes TNFα, LTα, LTβ complex, Fas ligand, CD70, CD40 ligand, CD30 ligand, nerve growth factor and more. A receptor β chain is shared by IL-3, IL-5 and GM-CSF. Homologous G-protein-coupled receptors are used by IL-8 and the chemokine family. The receptors for IFNα, β, ω, γ and IL-10 also

show homology, as do those for the TGFβ family of cytokines. Since IL-12 and IL-18 have overlapping activities, their receptors also may be related. These observations permit a rational organization of cytokines into families.

The advent of additional information concerning the relationship and location of cytokine genes, shared signal transduction pathways and the tertiary structure of cytokines may enable us to further classify newly discovered cytokines. Although we have always presumed that the most important cytokines and receptors have been discovered, we are repeatedly surprised by identification not only of novel cytokines and receptors, but whole families. Undoubtedly many more cytokines will be identified now that the genome has been mapped.

Why are cytokines important?
Recombinant cytokines provide useful laboratory probes for studying the cell biology of innate and adaptive immunity and inflammation. Cytokines are the major orchestrators of host defense processes and, as such, are involved in responses to exogenous and endogenous insults, repair and restoration of homeostasis. Microbial pathogens have operated on these principles far longer than immunologists and have been shown to produce variants of proinflammatory cytokines, their receptors and chemokine antagonists that subvert and suppress the host immune and inflammatory defenses. Deletion of these products reduces the pathogenicity of these viruses. In addition to their role in host defense, cytokines appear to play a major role in development and some of them may account for as yet unidentified embryonic inductive factors. The study of cytokines is also elucidating the mechanisms underlying pathophysiological processes. Cytokines mediate not only host responses to invading organisms, tumors and trauma, but also maintain our capacity for daily survival in our germ-laden environment. In fact, the development of more sensitive methods of detection is revealing the presence of detectable, but low levels of cytokines associated with a variety of binding proteins in the serum. This probably reflects the production of cytokines in response to the many nonpathogenic stimulants present in our conventional environment. Detection of cytokines in disease states may provide useful diagnostic tools. The therapeutic administration of pharmacological doses of cytokines or at times cytokine gene therapy is being used in a wide variety of infectious diseases and in immunocompromised patients with genetic defects, AIDS, autoimmune diseases and neoplasias. This has led to the development of numerous biotechnology firms which are also evaluating antagonists of cytokines for their anti-inflammatory effects.

Why a Cytokine Handbook?
Studies of cytokines have drawn scientists from a multiplicity of fields including immunologists, hematologists, molecular biologists, neurobiologists, cell biologists, biochemists, physiologists, and others. Consequently, the burgeoning field of cytokine research is unique and interdisciplinary. The chapters in this Handbook cover the structure and functions of cytokines, their genes, receptors, mechanisms of signal transduction and clinical applications. This fourth edition of *The Cytokine Handbook* is required, at this relatively early date, to keep up with rapid developments in these dynamic disciplines. All the chapters, and even this foreword, have been updated and numerous new chapters by internationally renowned experts have been added including the new Interleukins, 19–27. The new edition has been greatly reorganized with the addition of chapters to cover the more general topics of molecular genetics, signal transduction and common structural features of cytokines and their receptors. The cytokine chapters have been grouped based on the nature of their receptors. All the hematopoietin family members including growth hormone, prolactin, erythropoietin, ciliary neurotrophic factor and nerve growth factor as well as all of the interleukins are now included. There are now separate sections with multiple chapters on the interferon family, IL-1 family, TNF family, growth factors, the chemokine family and transforming growth factor family. Finally, there is an expanded section on the therapeutic

applications of cytokines and cytokine inhibitors. Thus, this fourth edition has been considerably expanded based on progress in cytokine research and on the fact that many additional intercellular mediators should be considered cytokines and included here. Consequently, *The Cytokine Handbook* provides us with the opportunity to keep up with the rapidly evolving studies of cytokines.

Acknowledgements
I am grateful for the critical input of Drs R. Neta, M. Grimm and S. Durum and the secretarial assistance of Ms Cheryl Fogle.

Joost J. Oppenheim
Laboratory of Molecular Immunoregulation
National Cancer Institute
Maryland, USA

REFERENCES

Bloom, B.R. and Bennet, B. (1966). Mechanism of a reaction *in vitro* associated with delayed-type hypersensitivity. *Science* **153**: 80–82.
Cohen, S., Bigazzi, P.E. and Yoshida, T. (1974). Similarities of T cell function in cell-mediated immunity and antibody production. *Cell. Immunol.* **12**: 150–159.
David, J.R., Al-Askari, S., Lawrence, H.S. and Thomas, L. (1964). Delayed hypersensitivity *In vitro*. *J. Immunol.* **93**: 264–273.
Dumonde, D.C., Wolstencroft, R.A., Panayi, G.S. *et al.* (1969). Lymphokines: non-antibody mediators of cellular immunity generated by lymphocyte activation. *Nature* **224**: 38–42.
George, M. and Vaughan, J.H. (1962). *In vitro* cell migration as a model for delayed hypersensitivity. *Proc. Soc. Exp. Biol. Med.* **111**: 514–521.
Gershon, R.K. and Kondo, K. (1971). Infectious immunological tolerance. *Immunology* **21**: 903–914.
Gershon, R.K., Gery, I. and Waksman, B.H. (1974). Suppressive effects of *in vivo* immunization on PHA responses *in vitro*. *J. Immunol.* **112**: 2215–221.
Gery, I., Gershon, R.K., and Waksman, B.H. (1971). Potentiation of cultured mouse thymocyte responses by factors released by peripheral leucocoytes. *J. Immunol.* **107**: 1778–1780.
Green, J.A., Cooperland, S.R. and Kibnick, S. (1969). Immune specific induction of interferon production in cultures of human blood lymphocytes. *Science* **164**: 1415–1417.
Grumet, M., Mauro, V., Burgoon, M.P. *et al.* (1991). Structure of a new nervous system glycoprotein, Nr-CAM, and its relationship to subgroups of neural cell adhesion molecules. *J. Cell. Biol.* **113**: 1399–1412.
Kasakura, S. and Lowenstein, L. (1965). A factor stimulating DNA synthesis derived from the medium of leucocoyte cultures. *Nature* **208**: 794–798.
Kolb, W.P. and Granger, G.A. (1968). Lymphocyte *in vitro* cytotoxicity: Characterization of human lymphotoxin. *Proc. Natl. Acad. Sci. USA* **61**: 1250–1255.
Lolekha, S., Dray, S. and Gotoff, S.P. (1970). Macrophage aggregation *in vitro*: A correlate of delayed hypersensitivity. *J. Immunol.* **104**: 296–304.
Mizel, S.B. and Farrar, J.F. (1979). Revised nomenclature for antigen non-specific T cell proliferation and helper factors. *Cell. Immunol.* **48**: 433–436.
Nathan, C.F., Karnovsky, M.L. and David, J.R. (1971). Alterations of macrophage functions by mediators from lymphocytes. *J. Exp. Med.* **133**: 1356–1376.
Rich, A.R. and Lewis, M.R. (1932). Nature of allergy in tuberculosis as revealed by tissue culture studies. *Bull. Johns Hopkins Hosp.* **50**: 115–131.
Ruddle, N.H. and Waksman, B.H. (1968). Cytotoxicity mediated by soluble antigen and lymphocytes in delayed hypersensitivity. *J. Exp. Med.* **128**: 1267–1279.
Waksman, B.H. and Matoltsy, M. (1958). The effect of tuberculin on peritoneal exudate cells of sensitized guinea pigs in surviving cell culture. *J. Immunol.* **81**: 220–234.
Ward, P.A., Remold, H.G. and David, J.R. (1969). Leukotactic factor produced by sensitized lymphocytes. *Science* **163**: 1079–1081

This Page Intentionally Left Blank

INDEX

(Note: page numbers in *italics* refer to figures and tables, those in **bold** refer to main discussions of topics. '*Plate*' refers to illustrations in Plate Section)