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Introduction 1

The mucosal immune system in the female reproductive tract has evolved to meet the unique requirements of dealing with colonization with commensal microorganisms, sexually transmitted bacterial and viral pathogens, allogeneic spermatozoa, and the immunologically distinct fetus. It is now established that the mucosal immune system is a distinct and separate component of the host's immune apparatus and differs from the lymphoid tissues in peripheral sites. Furthermore, despite some common features, the female genital tract mucosal system displays some distinct characteristics, which outlines its special role. Analysis of the female genital tract indicates that the key cells of the innate and adaptive immune systems are present and functionally responsive to antigens; however, there is a certain degree of compartmentalization within the tract. The identification of TLRs in the fallopian tubes, uterus, cervix, and vagina; the presence of ECs, macrophages, DCs, NK cells, and neutrophils throughout the reproductive tract; and the responsiveness of these cells to selected PAMPs indicate that the female reproductive tract has evolved to meet the challenges of STDs while at the same time supporting an immunologically distinct fetal placental unit. To meet these diverse challenges, the innate and adaptive immune systems in the female genital tract are precisely regulated not only by a network of cytokines and chemokines but also by the sex hormones estrogen and progesterone. The mechanisms that regulate this mucosal immune system are still only incompletely understood. This is due to the complexities and interactions of the female immune and endocrine systems as well as to difficulties of conducting experiments in this field.

During the last decade, there has also been an increasing need for further investigation in this area due to the rising prevalence of sexually transmitted diseases (STDs), among them the pandemics caused by the human immunodeficiency virus (HIV). Progress is being made in understanding how immune responses can best be stimulated at the genital tract mucosal level. On the basis of this information, the attempt to construct new mucosal vaccines specifically targeted to the genital tract is one of the ambitious goals of research in this field. Also, the blossoming of the field of reproductive medicine, with the tremendous increase in the number of infertile couples seeking to undergo assisted reproduction, has led to advances in characterizing immunological mechanisms and disturbances related to ovulation, conception, and pregnancy maintenance.

Understanding the specialty of the genital tract immune system is of critical importance, because STDs are and will continue to be a major health problem worldwide. Despite extensive efforts, only limited success has been achieved in dealing with a growing list of STDs. The role of immune factors in the control of genital viral and bacterial infections appears complex and needs further study, also with respect to the development of vaccines. Despite the recognition that innate immunity, as the first line of defense, and adaptive immunity, especially Th1 immune responses, play a critical role in preventing infection and in limiting viral

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replication, factors such as antimicrobials and TLRs that contribute to the mucosal response in the female genital tract have only recently begun to receive attention. Further studies are also needed to elucidate the relationship between mucosal immunity, the hormonal environment, and response to pathogen challenge.

More data must be collected on the mechanisms of immune evasion by several pathogens such as HSV, *N. gonorrhoeae*, and *Chlamydia*. While considerable information can be obtained from animal experiments, important differences in the physiology of reproduction and the immune system result in the need for studies in humans. Further knowledge on female tract immunology will also impact on immunological approaches to contraception, immunological infertility, and the immunological aspects of pregnancy. This will introduce new options not only for diagnostics but also for treatment of pregnancy complications such as preeclampsia, preterm birth, and early pregnancy loss as well as infertility.

The objective of this book is to systematically review and discuss recent advances in immunology of the female genital tract. The emphasis hereby lies on the evaluation of studies concerning the basics of female reproductive immunology, research on immunology of the most important genital infections and vaccination strategies, immunological principles at the fetomaternal interface during normal pregnancy and its complications, and immunological data on infertility and immunocontraception.

The second chapter in this book gives a brief introduction to the basic principles of human innate and acquired immunity and mucosal immunology, while the third aims to define the mucosal immune system in the female reproductive tract. The focus thereby is on identification of what is known about the humoral and cellular factors of this particular mucosal immune system and definition of the regulatory influences of sex hormones and cytokines. The unique immunological characteristics of the female genital tract are then considered with respect to the design of mucosal vaccines for protection against microbial disease.

The fourth chapter deals with the most important infections of the female genital tract and describes the latest results regarding innate and adaptive immune responses and vaccine development for each infection. These are the following:

- Viral infections
  - *Herpes simplex virus (HSV)*
  - *Human immunodeficiency virus (HIV)*
  - *Human papillomavirus (HPV)*
- Bacterial infections
  - *Neisseria gonorrhoeae*
  - *Chlamydia trachomatis*
  - *Bacterial vaginosis (BV)*
- Mycoses
  - *Candida albicans*
- Parasites
  - *Trichomonas vaginalis*

Finally, the fifth chapter reports on different important areas of immunology in reproductive medicine. Pregnancy involves maternal tolerance of the semiallogenic histoincompatible fetus and is characterized by the enhancement of the innate immune system and suppression of the adaptive immune response, probably with progesterone as the important regulator. In contrast to normal pregnancy, improper immune responses and an unbalanced cytokine network may characterize implantation failures, pregnancy loss, and obstetric complications. These are the presence of elevated Th1/Th2 cell ratios, high concentrations of Th1 cytokines, elevated NK cell cytotoxicity and levels, and emergence of various autoantibodies. Immunological approaches need to be investigated and evaluated further with respect to widening of treatment options by modification of immune responses.

After describing immunological principles at the fetomaternal interface during normal pregnancy and labor, different disturbances in maternal–fetal interactions and their immunological background are discussed. These are:

- Preeclampsia
- Preterm labor/preterm birth
- Fetal growth retardation
- Early pregnancy loss

Furthermore, the topic of infertility is further elucidated from an immunological point of view. Remarks on the latest developments in immunocontraception conclude this chapter.

Fundamentals of Immunology

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Contents

2.1 Concepts of Innate and Specific Immunity

The term immunity derives from the Latin word *immunitas*, which stands for the privilege of the Roman senators to be protected from legal punishment or exempted from certain public duties. Initially, immunity was described as protection against illness, especially infectious illness. In modern times immunity has been more exactly defined as reactions of the body against foreign or altered self substances.

The immune system has evolved to provide appropriate defense systems at various levels of innate (nonspecific) and acquired (specific) immune responses. Innate immunity is the ancient part of the host defense mechanisms and lies behind most inflammatory responses. Acquired immunity can provide specific recognition of foreign antigens, an immunological memory of infection, and pathogen-specific adaptor proteins. However, the adaptive immune response is also responsible for allergy, autoimmunity, and the rejection of tissue grafts.

In most cases, components of both systems interact to create an appropriate immune response to an infectious agent. But it is not only the protection from penetration by foreign or modified cells but also the elimination of old and deficient cells which characterizes a functioning immune system.

2.1.1 The Innate Immune Response to Infectious Agents

Recognized as the first line of defense, innate immunity consists of different mechanisms which are already available before exposition with pathogens or unknown molecules and do not need prior activation or induction. Innate immune responses do not change in type or magnitude if there is more than one encounter with the same antigen, and differences between unknown substances cannot be distinguished. In the following, a short overview of the different factors of innate immunity is given (Table 2.1).

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Table 2.1 Factors of the innate immune system

Mechanical and chemical barriers
Skin, mucosal surfaces
Enzymes (lysozyme), peptides (defensins), fatty acids, acidic pH, etc.
Cellular factors
Mononuclear phagocytes (blood monocytes/tissue macrophages)
Granulocytes
Dendritic cells
Mast cells
Natural killer cells
Humoral factors
Complement
Acute-phase proteins
Interferons

M. Wirth 2006, Personal communication

Table 2.2 Localization of phagocytic cells

Localization	Phagocytic cells
Blood	Monocytes
	Neutrophils (eosinophils. basophils)
Lung	Alveolar macrophages
Liver	Kupffer cells
Brain	Microglia
Bone	Osteoclasts
Kidney	Mesangial cells in glomeruli
Joint	Synovial A cells

M. Wirth 2006, Personal communication

Preventing microorganisms from gaining access to the body is achieved by mechanical barriers such as the skin and surface epithelia. These are also equipped with additional chemical features including fatty acids in the skin, low pH in the stomach, or antibacterial enzymes in saliva, for example. Once the pathogen has crossed the epithelial barrier, cellular effector mechanisms involving granulocytes and mononuclear phagocytes are activated to eliminate the intruder by phagocytosis.

To recognize foreign structures that are not normally found in the host, the innate immune system relies on conserved germline-encoded receptors that recognize conserved pathogen-associated molecular patterns (PAMPs) found in groups of microorganisms. These are essential conserved products produced by microorganisms but not by the host, such as lipopolysaccharide (LPS) in the outer membrane of gram-negative bacteria and peptidoglycan membrane components of gram-positive bacteria. Recognition of these molecular structures allows the immune system to distinguish infectious nonself from noninfectious self.

Among receptors for PAMPs which are expressed by cells of the innate immune system, Toll-like receptors (TLRs) are of major importance. Signaling through TLRs in response to PAMPs leads to recruitment, differentiation, and activation of other immune cells and production of antimicrobial factors that kill invading microbes as well as link innate and acquired immunity. Studies have found 10 different subtypes of mammalian TLRs. Among the cells that bear innate immune or germline-encoded recognition are mononuclear phagocytes, dendritic cells (DCs), mast cells, granulocytes, natural killer (NK) cells, and epithelial cells.

2.1.1.1 Cellular Factors

Phagocytic cells are located at strategically important locations in the organism (Table 2.2) and are all derived from pluripotent stem cells of the bone marrow.

Monocytes circulating in blood vessels and macrophages in other tissues like the lung, liver, and lymph nodes as well as granulocytes are capable of killing microorganisms. After activation by cytokines, especially interferon-γ (IFN-γ), macrophages produce toxic effector molecules such as reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI) which are interactively able to kill the pathogen. RNI have proved to be the most effective defense mechanism in murine macrophages but also an increasing amount of studies supports RNI production by human macrophages. Lysosomal enzymes within the phagosome or depletion of intraphagosomal iron function as other mechanisms of phagocytic cells to kill intracellular pathogens.

The group of granulocytes consists of neutrophils, eosinophils, and basophils, but the uptake and intracellular killing of microorganisms is in the first place the task of neutrophils. By expressing Fc receptors for immunoglobulin (Ig)

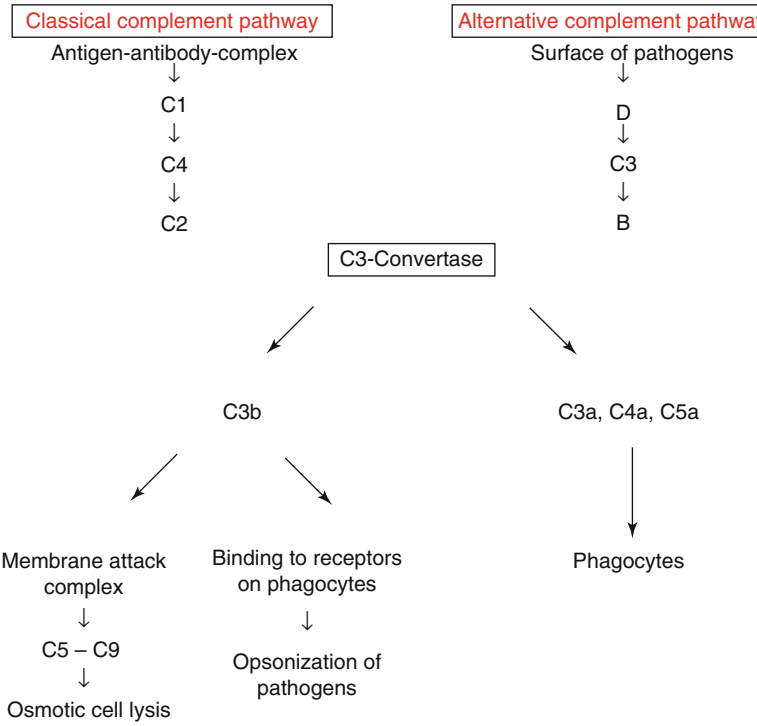

Fig. 2.1 Components and effector mechanisms of the complement system (Adapted from Janeway CA Jr., Travers P. Immunologie. 2nd ed. Heidelberg: Berlin: Oxford; Spektrum Akademischer Verlag; 1997)

G (CD16) and receptors for activated complement factors (C3b), neutrophils can phagocyte pathogens coated by antibodies or complement factors. Neutrophils are able to release azurophil granule with myeloperoxidase and lysozyme, specific granule with lactoferrin or alkaline phosphatase as well as superoxide radicals, which leads to an inflammatory reaction of tissue. Extracellular killing of large parasites such as helminths is performed by eosinophils. They also provide surface receptors for complement factor C3b and release their granule with major basic protein (MBP), cationic protein, and anti-inflammatory enzymes. Basophils play an important role in allergic reactions and in immune responses against parasites where they release mediators such as histamine or heparin and chemotactic factors and therefore induce an anaphylactic reaction. They have surface receptors for both IgE and activated complement factors C3a and C5a.

Other cellular components of the innate immunity are NK cells which are large granular lymphocytes comprising 5–10 % of circulating peripheral lymphocytes. They express surface receptors for IgG (CD16) as well as T cell markers such as CD8 and are able to mediate antibody-dependent cellular cytotoxicity (ADCC). Thereby, they are able to destroy target cells such as malignantly transformed cells or virus-infected cells by binding these antibody-loaded cells to the Fc receptor of NK cells via the Fc region of the antibody.

2.1.1.2 Humoral Factors

The complement system is a multicomponent triggered enzyme cascade (Fig. 2.1). Activation results in direct killing of viruses and bacteria as well as marking microorganisms for ingestion by phagocytic cells.

Complement activation can start via the classical pathway with Igs in complex with antigens binding to complement protein C1. The alternative pathway beginning with the binding of complement factor C3b on different activated bacterial surfaces is initiated in the absence of antibodies and therefore is a more rapid humoral mechanism. A third pathway, the lectin pathway, is initiated by

the binding of mannose-binding lectin (MBL) to carbohydrate patterns on microorganisms. This activates MBL-associated serine proteases to cleave C2 and C4, generating the C3 convertase C4bC2a and cleavage of C3. All pathways lead to the splitting and activation of factor C3 whose activated fragments enable the opsonization of antigens on bacteria surfaces. The terminal components C5–C9 form a membrane attack complex which enables the osmotic lysis of the pathogen.

Other humoral factors of the innate immune system involve IFNs and acute-phase proteins. IFN-α produced by leukocytes and IFN-β synthesized by fibroblasts and other cell types block viral replication in virus-infected cells. C-reactive protein as the most common representative of acute-phase proteins promotes the binding of complement to bacteria and facilitates their phagocytosis.

2.1.2 Specific Acquired Immunity

Following interaction of the intruding microorganism with effector mechanisms of the innate immune system, components of the specific or acquired immune system are activated. Acquired immunity is characterized by recognition of specific antigenic determinants. The following responses to the same antigen are specific and quantitatively and qualitatively different from the primary response. This specificity is achieved through the use of clonally distributed antigen receptors, i.e., surface Ig on antibody-producing B lymphocytes and T cell receptors (TCR) on the surface of T lymphocytes. Another feature of the adaptive immune system is that it develops memory which allows a faster response of specific effector cells when encountering the relevant antigen a second time.

Responding to antigens is either realized by producing specific antibodies (humoral immunity) or direct specific-lymphocyte contact with host cells expressing foreign antigenic peptides (cell-mediated immunity). The cell-mediated response needs the cooperation of different subclasses of T cells, macrophages, and perhaps NK cells. Humoral responses involve the interaction of B cells, T cells, and antigen-presenting cells (APCs).

Table 2.3 MHC classes with examples for encoding proteins

MHC class Ia	HLA-A, HLA-B, HLA-C
MHC class Ib	HLA-E, HLA-F, HLA-G
MHC class II	HLA-DP, HLA-DQ, HLA-DR
MHC class III	Complement components, TNF-α, TNF-β

M. Wirth 2006, Personal communication

2.1.2.1 The Major Histocompatibility Complex

T and B lymphocytes need a system to distinguish between "self" and "nonself," which is the function of the major histocompatibility complex (MHC). This group of genes encodes for several proteins, also called human leukocyte antigens (HLA) (Table 2.3). MHC class I molecules, which include HLA-A, HLA-B, and HLA-C, are expressed by all nucleated cells whereas MHC class II molecules including HLA-DR, HLA-DP, and HLA-DQ are expressed by APCs and B and T lymphocytes.

2.1.2.2 B Lymphocytes

After the process of B-cell differentiation in the bone marrow, each mature B cell bears a surface receptor, an Ig, which is different in its antigen specificity from all other B cells. These mature but naive B cells circulate in the blood, lymph, and secondary lymphoid organs waiting to encounter antigen. By interaction with antigen, B cells differentiate into antigen-specific memory B cells and effector plasma cells which generate large amounts of soluble versions of the membrane-bound Ig.

This clonal selection hypothesis explains why subsequent responses to the same antigen are more effective and long-lasting than the initial response. In the first encounter with antigen, a primary antibody response is generated; later, a reencounter with the same antigen causes a more rapid secondary response, producing high levels of antibodies with a strong affinity for the target antigen. This process is also exploited in prophylactic vaccination.

The primary humoral immune response involves generation of IgM class antibodies, whereas the secondary and all subsequent responses to the same antigen may be of IgG, IgE,

Table 2.4 The different classes of immunoglobulins

	IgG	IgA	IgM	IgD	IgE
Form	Monomer	Monomer Dimer	Pentamer Hexamer	Monomer	Monomer
Subclasses	G1,G2,G3,G4	A1. A2	–	–	–
Percent of Ig	75–85	7–15	5–10	0.3	0.019
Binds to	Macrophages NK cells Neutrophils	Lymphocytes	Lymphocytes	–	Mast cells Basophils B cells
Complement fixation	Classical	Alternative	Classical	–	–
Cross placenta	Yes	–	–	–	–

M. Wirth 2006, Personal communication

or IgA subclasses, depending on specific location. Proliferation and differentiation of B cells as well as Ig isotype class switching are driven by cytokines, especially by interleukins (IL)-4 and -5. The differences between the Ig subclasses are shown in Table 2.4. In addition to their unique role as antibody-producing plasma cells, B cells have the capacity to present antigen to T lymphocytes. Upon binding of antigen to membrane-bound Ig, antigen–antibody complexes are internalized and degraded. Antigen-derived peptides are then introduced to MHC-II-dependent pathways and can be presented to peptide-specific CD4+ T cells.

2.1.2.3 T Lymphocytes

In contrast, T cells only recognize antigen when it is presented by appropriate MHC molecules on APC such as DCs or macrophages. During their differentiation in the thymus, T lymphocytes learn to recognize MHC molecules and develop the cluster of differentiation (CD) 4 and 8 surface receptors which mark them as CD4+ T helper cells or CD8+ cytotoxic T cells (CTL). Antigenic peptides presented to T cells by MHC class I molecules stimulate the CD8+ T cells whose primary function is to destroy intracellular pathogens. Peptides presented by MHC class II molecules stimulate the CD4+ T cells which are able to eliminate both intracellular and extracellular pathogens. They produce various cytokines required for the activation of leukocytes and are therefore also termed T helper (Th) cells.

Activation of T cells requires signaling mediated through both the TCR and costimulatory receptor–ligand interactions which involve the costimulatory molecules CD80 (B7.1) and CD86 (B7.2) on APCs. These can bind to T cell surface molecule CD28 and CD125 (CTLA-4).

As CD4+ Th cells produce different cytokines upon antigenic stimulation to activate B cells and macrophages, they can be divided into the three subpopulations of Th1, Th2, and Th17 cells. Th1 cells are characterized by secretion of IFN-γ and IL-2; Th2 cells produce IL-4, IL-5, and IL-10; and Th17 cells release IL-17. Th2 cells are therefore important for the induction of humoral immune responses by controlling B-cell activation, Th1 cells initiate cell-mediated immune responses by activating macrophages by IFN-γ and CD8+ T cells by IL-2, and Th17 cells activate neutrophils (667, Table 2.5). The differentiation of these subsets from Th0 precursor cells is driven by cytokines, especially by IL- 12/IL-18 and IL-4, respectively.

A fourth category of T cells, regulatory T cells (Tregs) with the phenotype CD4+ CD25+, usually secretes IL-10 and tumor growth factor-β (TGF-β). Cells with this phenotype are thought to recognize self-antigens and function to prevent autoimmunity, but they also regulate responses to exogenous antigens and have been implicated in chronic and immunopathologic viral infections.

CTLs are able to eliminate virus-infected cells or tumor cells by lysis or apoptosis. Cytotoxicity is mediated by pore-forming proteins (perforins) and enzymes (granzymes) that perforate the target cell. CTLs can also trigger apoptosis,

Table 2.5 The role of Th1, Th2, and Th17 cells in immunity

Activation of cytotoxic T cells → protection against viruses	B-cell maturation → protection against extracellular microbes, virions, and helminths
Macrophage activation → protection against intracellular microbes	Ig class switch to IgE (mast cell, basophil, eosinophil) → protection against helminthes
Ig class switch to IgG (complement activation/ opsonization) → protection against extracellular microbes	Ig class switch to IgG (neutralization) → protection against virions, toxins
Th1 activation → protection against all microbes and viruses	Ig class switch to IgA (mucosa) → protection against many pathogens
	Eosinophil activation → protection against helminths

Adapted from Kaufmann SHE, Kabelitz D. The immune response to infectious agents. In: Kaufmann SHE, Kabelitz D, editors. Immunology of infection. Methods in microbiology, vol. 32. 2nd ed. London: Academic Press; 2002. p. 1–20

i.e., programmed cell death, in target cells through receptor–ligand interaction. Upon activation, CTLs are induced to express Fas ligand (FasL) which interacts with the corresponding receptor Fas expressed on virus-infected target cells.

The division of the immune system into innate and specific immunity (Table 2.6) does not mean the strict separation of both when encountering pathogens. Instead, it is required that both systems closely cooperate and that components of both activate each other. Over the recent years, it has become increasingly clear that the two systems cannot be seen separately and that the innate immune system is even instrumental for the development of the adaptive immune response.

2.2 Mucosal Immunology

The immune system can be divided into two compartments that display considerable functional independence: on the one hand, the systemic compartment represented by the bone marrow, spleen, and lymph nodes and, on the other hand, the mucosal compartment represented by lymphoid tissues in mucosae and external secretory glands. Numbers and types of cells involved in immune responses and their soluble products, primarily antibodies, are remarkably different in the mucosal and systemic compartments of the immune system, which are now further elucidated.

A distinct immune system at mucosal surfaces in humans was first presumed during the times of Paul Ehrlich in the nineteenth century. Further anatomical and biological studies of a common mucosa-associated immune system go back to the 1970s where Tomasi described the function of secretory (S)-IgA and cellular immune mechanisms as components of a mucosa-associated immune system.

Several hundred square meters comprise the surface areas of mucosal membranes where antigens from ingested food or inhaled air and resident pathogens represent the most important exogenous stimulants. Due to the high antigen load of mucosal surfaces, the mucosal immune system exhibits immunological hyporesponsiveness or unresponsiveness to most antigens. On the other hand, it must also be capable of inducing effective cell-mediated and antibody-mediated immune responses toward selected antigens. To meet this task, mucosal surfaces possess a unique immune system that tightly controls the balance between responsiveness and nonresponsiveness (tolerance). Besides mechanical barriers, humoral factors such as lysozyme, peroxidase, and specific antibodies as well as cellular mechanisms contribute to the protection of mucosal surfaces (Table 2.7).

Immune responses generated by organized lymphoid structures in the mucosa-associated lymphoreticular tissue (MALT) result in the development of B cells capable of producing antigen-specific Igs that can reach the draining lymph nodes and other mucosal tissues where they differentiate into plasma cells. A second major outcome of the entry of antigen and antigen presentation by DCs is the activation and differentiation of T cells that can subsequently

Table 2.6 The concepts of innate and specific acquired immunity

	Innate immune system	Adaptive immune system
General characteristics	First line of defense	Specific recognition of foreign antigens
	No specificity and no adaptation following antigen exposure	Immunological memory
		Responsible for allergy. autoimmunity, rejection of tissue grafts
Physical and chemical barriers	Skin	Immune systems of skin and mucosal membranes
	Mucosal membranes	Antibodies in secretions
Circulating molecules	Complement	Antibodies
Cellular factors	Macrophages/monocytes	Lymphocytes
	Granulocytes	
	Natural killer cells	
Soluble mediators effective on other cells	Cytokines like α- and β-interferons, tumor necrosis factor (derived from macrophages)	Cytokines like γ-interferons (derived from lymphocytes)
Receptors	Genes encoded in germline DNA	Encoded in gene segments
	No gene rearrangement	Rearrangement necessary
Recognition	Conserved molecular patterns (PAMPs)	Details of molecular structure (proteins. peptides)
Self-nonself discrimination	Perfect (selected over evolutionary time)	Not perfect (selected in individual somatic cells)
Response	Immediate activation of effectors	Delayed activation of effectors

Adapted from Janeway CA Jr., Travers P. Immunologie. 2nd ed. Heidelberg: Berlin: Oxford; Spektrum Akademischer Verlag; 1997

Table 2.7 Protection of mucosal surfaces

Mechanical barriers and peristalsis
Desquamation of epithelial cells with attached microorganisms
Humoral factors: Mucin, acids, lysozyme, lactoferrin, peroxidase system antimicrobial proteins, interferon-α. Complement specific antibodies: IgA ≫ IgG > IgM
Cellular factors: Phagocytic cells, T cells, NK cells

Adapted from Kutteh et al. Reproductive Immunology. Ann Arbor: Blackwell Science; 1996. p. 28–51

migrate out of the MALT and reach mucosal as well as peripheral non-mucosal tissues.

The mucosal immune system is structurally and functionally divided into sites for antigen uptake and processing at inductive sites on the one hand and effector sites engaging lymphocytes, granulocytes, and mast cells on the other hand. Besides the nasal-associated lymphoreticular tissue (NALT), the gut-associated lymphoreticular tissue (GALT) is the prototype of MALT and possesses APCs, T lymphocytes, and IgA-committed B cells.

2.2.1 S-IgA as the Major Ig Subclass in the Mucosal Immune System

Igs of all isotypes have been detected in various human external secretions. The predominant Ig isotype in normal human serum is IgG, followed by IgA and IgM. In contrast to serum, the major isotype in human excretions such as saliva, tears, bile, urine, and milk is IgA. IgA is the most important subclass of Igs that can actively and efficiently be secreted through epithelia. Due to the size of mucosal surfaces, the total amount of daily IgA production was quantified as 66 mg/kg body weight, which is more than twice the concentration of IgG. Approximately 1,500 mg/day IgA is produced systemically in the bone marrow, lymph nodes, or spleen but twice as much IgA is released in the mucosal immune system.

Most of the serum IgA is found in a monomeric form with two heavy and two light chains whereas S-IgA is mainly polymeric with presence of a J chain and secretory component (SC). IgA is produced by plasma cells in the lamina

Table 2.8 Effector functions of secretory and serum IgA

	Secretory-IgA	Serum IgA
Molecular form	Polymeric	Monomeric
Subclass	IgA1 ≥ IgA2	IgA1 >>> IgA2
SC- mediated transport into secretions	Yes	No
J-chain expression	Yes	Mostly no
Origin of precursor cells	Bone marrow, no circulation	Peyer's patches, IgA cells in circulation
Neutralization of antigens	Yes	Yes
Inhibition of bacterial adherence	Yes	?
Loss of bacterial plasmid	Yes	?
Inhibition from antigen uptake from mucosa	Yes	No
Enhancement of innate factors	Yes	Yes (?)
Suppression of inflammatory effects (phagocytosis, lysis, NK cell activity, etc.)	Yes	Yes

Adapted from Kutteh et al. Reproductive Immunology. Ann Arbor: Blackwell Science; 1996. p. 28–51

propria and combined with the SC, a glycoprotein expressed on the surface of mucosal epithelial cells (ECs). This protein then handles the active transport of polymeric IgA into the intestinal lumen where IgA is released by proteolytic cleavage again. Investigations concerning the origin of S-IgA have demonstrated that it is produced locally at mucosal sites and is not derived to a significant degree from the circulation.

The two different molecular forms of IgA also show different effector functions, which is illustrated in Table 2.8.

IgA can also be divided into two subclasses IgA1 and IgA2 which differ in primary structure, carbohydrate composition, and their sensitiveness to bacterial proteases. Neisseria gonorrhoeae, for instance, is a producer of extra cellular proteases, specific for IgA which constitutes its most important phatogen factor, which is specific for IgA1. In serum, monomeric IgA1 predominates over IgA2 whereas in external secretions

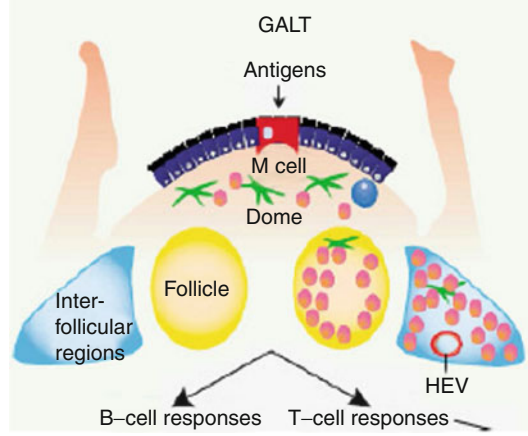

Fig. 2.2 Inductive site of the GALT (Neurath MF, Finotto S, Glimcher LH. The role of Th1/Th2 polarization in mucosal immunity. Nat Med. 2002;8:567–73)

almost exclusively polymeric forms of approximately equal proportions of IgA1 and IgA2 are found. Furthermore, specific antibodies to viral antigens, including HIV, are often found in the IgA1 subclass, whereas IgA2 antibodies in external secretions are associated with specificity for common structural microbial antigens as LPS and lipoteichoic acid.

2.2.2 Inductive Sites of the Mucosal Immune System: GALT

The primary inductive sites for mucosal immune responses are organized lymphoid aggregates such as Peyer's patches in the wall of the intestine or tonsils in the uppevr respiratory tract. The Peyer's patches of the GALT consist of a follicle-associated epithelium with specialized ECs known as membranovus epithelial (M) cells, a subepithelial dome overlying B-cell follicles, and interfollicular regions enriched in T cells (1528, Fig. 2.2). Following ingestion, antigens and microorganisms are transported from the gut lumen to the dome region through specialized M cells where they encounter APCs such as DCs leading to cognate interactions between APCs and T cells. DCs can also migrate to the interfollicular regions which are enriched with T cells and containing high endothelial venules (HEV) and efferent lymphatics to initiate immune responses upon antigen uptake. DCs as the APCs bind bacterial products

with their TLRs, process antigen as relative immature cells, and then migrate to the T cell region and present antigen to naive T cells. There they have the properties of mature and immunogenic DCs with high surface expression of costimulatory molecules such as CD40, CD80, and CD86 and adhesion molecules such as CD44.

After antigen uptake via M cells, B cells are induced to switch into IgA-secreting cells. Following IgA switch and affinity maturation, B cells migrate from the Peyer's patches to the mesenteric lymph node via efferent lymphatic vessels and finally to the lamina propria where they undergo terminal differentiation to plasma cells. Peyer's patches are an enriched source of IgA precursor cells capable of lodging in the recipient's gut as well as in other glands and mucosal tissue. Depending on the type of antigen and the duration of stimulation, ingestion of pathogens induce local and systemic immune responses with parallel appearance of specific S-IgA in saliva, milk, and tears, for example. The production of IgA is therefore induced in lymphoid follicles such as the Peyer's patches from where the cells recirculate through lymph and blood to diffusely populate other mucosal tissues and exocrine glands where terminal differentiation into IgA plasma cells under the influence of locally produced cytokines occurs.

This provides the mucosal immune system with the ability to induce responses at sites that are distant from the immediate inductive environment, or even in different mucosal tissues. This has led to the concept of generalized functioning of mucosal tissues with some cross talk between them. Substantial dissemination of primed immune cells from GALT to exocrine effector sites beyond the gut is also the rationale for many desired oral vaccines.

2.2.3 Effector Sites of the Mucosal Immune System, in the Example of the GALT

Following induction in the MALT, mature lymphocytes leave the inductive sites and migrate to the effector sites such as the lamina propria where they can induce proinflammatory as well as suppressive immune responses. Effector mechanisms that protect mucosal surfaces include CTLs and effector CD4+ T cells for cytokine production and IgA response. Lamina propria T cells are mainly CD4+ Th cells (60–70 %), the majority of which also express the TCR, just as in peripheral blood. However, lamina propria T cells are in a more activated state than blood lymphocytes and have a mature or memory phenotype, indicated by the surface markers CD44, CD62, and CD45RO+.

Cytotoxic CD8+ T cells account for about 30–40 % of T cells in the lamina propria. They control the level of viral infection and have a cellular memory. A more restricted T-cell population, the intraepithelial lymphocytes (IEL), mainly CD8+ T cells, may play a role in maintenance of epithelial integrity and in class switching to IgA. Cytologically, they are T lymphocytes but their function is equivalent to NK cells of the innate immunity.

Besides an inflammatory phenotype T cells can adopt immunosuppressive function. These cells have been termed Treg cells, and it is currently not clear if these cells are identical cell types or different immunoregulatory cells. Treg cells can actively inhibit activation or differentiation of other T cells and also express the CD25 marker besides their CD4 marker. Treg cells have been shown to produce large amounts of IL-10 and/or TGF-β, and their immunosuppressive properties are most likely explained by the ability of these cytokines to inhibit APC function and to mediate direct antiproliferative effects on T cells.

Another task which is presumably performed by T cells is the constant distinguishing of harmless antigens in food and on commensal bacteria from pathogenic microbes. Oral tolerance is defined as the induction of a state of systemic immune nonresponsiveness to orally administered antigen upon subsequent antigen challenge. This mechanism seems to prevent the development of an immune reaction or allergy against intestinal intraluminal antigens. T cells appear to be the major target of tolerance and the reduction in antibody responses after antigen exposition is due to the reduction in T helper activity rather than to a tolerization of B cell directly. In addition to active suppression by Treg cells, tolerance is also maintained by deletion and anergy of T cells specific for luminal antigens. Deletion of specific T cells occurs by apoptosis whereas anergy of mucosal T

Table 2.9 Cytokine help for the regulation of mucosal immunoglobulin response

Th subset	Cytokine production	Effect on IgA response
Th1	IL-2	Synergizes with IL-5/ TGF-β → IgA synthesis
	IFN-γ	Ig switch to IgG2a
	Lymphotoxin β	Development of Peyer's patches
Th2	IL-4, IL-5	Differentiation to plasma cells
	IL-6	IgA synthesis
	IL-10	IgA synthesis
Th3	TGF-β	IgA isotype switching
TR1	IL-10, TGF-β	Suppression of immune responses
		Downregulation of Th1

Adapted from Wittig BM, Zeitz M. The gut as an organ of immunology. Int J Colorectal Dis. 2003;18:181–7

cells is believed to be induced when cells are stimulated without proper costimulatory molecules.

2.2.4 Cytokine Regulation of the Gut Mucosal Immune Response

The differentiation into Th cells and Treg cells in the mucosa results in secretion of proinflammatory cytokines such as IFN-γ and TNF-α by Th1 cells; Th2 cells secrete IL-4, IL-5, IL-6, IL-10, and IL-13 and promote IgA expression, Treg cells secrete TGF-β, and Treg produce predominantly IL-10 (Table 2.9). Several reports suggest that the level and type of costimulation a naive T cell receives influences whether Th1, Th2, or Th17 cells develop. Further, different types of APCs may selectively trigger either Th1 or Th2 responses; however, the same APC cell can function equally well for inducing a Th1 or Th2 response.

Further Reading

Concepts of Innate and Specific Immunity

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