Effects of Early Environment on Mucosal Immunologic Homeostasis, Subsequent Immune Responses and Disease Outcome

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Abstract

During the neonatal period, the mammalian host is exposed through mucosal surfaces for the first time to a plethora of environmental macromolecules and microbial agents. The neonatal mucosa is endowed with all major elements of innate and adaptive immunologic repertoire. Rudimentary Peyer's patches and mucosal lymphoid follicles expressing HLA-DR+ and CD4+ cells can be observed as early as 10–11 weeks of gestation. CD5+ and IgA+ B cells can be detected in Peyer's patches by 16–18 weeks. CD7+ CD3+ T lymphocytes have been observed in fetal Peyer's patches, epithelial surfaces as well as in the lamina propria. Interestingly, however, the early neonatal period is also characterized by a relative deficiency in antigen-presenting cell functions, altered cell-mediated immune responses, and a relative increase in apoptosis and eosinophilic responses. After birth, each human being may be colonized by over 100 trillion bacteria, representing over 500 bacterial species. The ratio of bacterial to human cells in a normal adult may exceed 10:1. The nature and the species of microflora acquired in the first few months of life is determined by many factors including, external environmental microflora, introduction of cow’s milk, use of antibiotics and immunomodulatory agents, and use of breastfeeding. Recent Investigations have shown that the nature of mucosal microflora acquired in early infancy determines the outcome of mucosal inflammation and the subsequent development of mucosal disease, autoimmunity and allergic disorders later in life. It appears that altered mucosal microflora in early childhood alters signaling reactions which determine...
T cell differentiation and/or the induction of tolerance. Reduced Th1 and increased Th2 cytokine expression in the respiratory tract associated with increased allergic disease has been correlated with reduced exposure to microbial agents associated with Th1 responses. In contrast, reduced exposure to helminthes in the gut associated with reduced Th2 expression appears to correlate well with dominant Th1 cytokine expression and inflammatory bowel disease. These observations suggest that the nature of interaction between the external environment and the mucosal tissues in the early neonatal period and infancy may be critical in directing and controlling the expression of disease-specific responses in later life.

Introduction

In his fascinating science fiction novel ‘Prey’, Michael Crichton [1] proposes a very provocative view of distributed systems, past learning and external environment, and their impact on the course of biologic evolution. He states, ‘The first life shows up four billion years ago as single-cell creatures. Nothing changes for the next two billion years. Then nuclei appear in the cells. Things start to pick up. Only a few hundred million years later, multicellular organisms. A few hundred million years after that, explosive diversity of life. And more diversity. By a couple of hundred million years ago there are large plants and animals, complex creatures, dinosaurs. In all this, man’s a latecomer: four million years ago, upright apes. Two million years ago, early human ancestors. Thirty-five thousand years ago, cave paintings.

The acceleration was dramatic. If you compressed the history of life on earth into twenty-four hours, then multicellular organisms appeared in the last twelve hours, dinosaurs in the last hour, the earliest men in the last forty seconds, and modern men less than one second ago.

It had taken two billion years for primitive cells to incorporate a nucleus, the first step toward complexity. But it had taken on 200 million years – one-tenth of the time – to evolve multicellular animals. And it took only four million years to go from small-brained apes with crude bone tools to modern man and genetic engineering. That was how fast the pace had increased’.

Although the scientific accuracy of the temporal events proposed in this science fiction setting remains to be determined, it is important to recognize that evolution of mammalian immunologic functions has also been repeatedly shaped by past learning and external environmental conditions in a manner similar to evolution of life itself. The greatest impact has been on the evolution of innate and adaptive immunologic defenses, acquisition of a symbiotic relationship with highly selected microbial flora in mucosal surfaces, and on a balanced interaction between the host and the external microbial environment. The outcome of such interactions has ultimately determined the health and the survival of the species. During the past few centuries, these biologic interactive
processes have been drastically influenced by the evolution of human societal culture associated with continuing introduction of numerous man-made modalities designed to improve human life and living conditions on earth.

This review will attempt to relate microbiologic, environmental and host mucosal immunologic factors to the mechanisms of prevention and control of human diseases, as well as to the possible evolution of several newly acquired diseases in man, with well-defined or possible immunologic basis.

**Host Development: Mucosal Defenses**

Over the past several million years of mammalian evolution, complex mechanisms of systemic and mucosal defenses have evolved. These include mucosal surfaces and cutaneous tissues and their barrier elements, innate immune functions, and adaptive immunity.

The nonspecific mucosal barriers consist of intact mucosal surfaces, gastrointestinal digestive enzymes, mucin, glycoproteins and several other mucosal repair and protective peptides such as, the trefoil factors, paneth cells, and defensins [2–5].

**Innate Immunity**

The major effector mechanisms of innate immunity consist of pathogen recognition receptors (PRRs), designed specifically to recognize unique pathogen-associated molecular patterns (PAMPs). Other effector mechanisms include, several antimicrobial peptides, phyocytes, dendritic cells (DCs), and alternate pathway complement products. It is believed that innate immunologic mechanisms have appeared long before the development of specific adaptive immunity and some form of innate immunity may exist in all multicellular organisms [6, 7].

Innate immune recognition appears to be mediated by germ-line-encoded receptors, in which the specificity of each receptor is genetically predetermined. The recognition receptors appear to have evolved by natural selection with defined, albeit limited specificity for infectious microorganisms. It has been proposed that unlike adaptive immunity, the PRR in innate immunity recognize limited, but highly conserved antigens or antigenic determinants (PAMPs) present in many organisms [8]. These include bacterial lipopolysaccharide (LPS), peptidoglycan, mannans, microbial DNA, 8sRNA, and other microbial determinants. It is estimated that the total number of PRRs in the innate immune system is very small and limited at the most to several hundred (10^2–3). On the other hand, the number of different somatically generated immunoglobulin and T and B cell receptors in fully developed adaptive immune responses is estimated to be in excess of 10^{14} and 10^{18}, respectively [9].
Pathogen Recognition Receptors

Important pathogen recognition-bearing structures identified to date are described in table 1. Such receptors are expressed on a variety of mucosal and other body tissues, including macrophages, DCs, and B cells. These receptors belong to several distinct protein families and function quite independently.

Secreted receptors include mannan-binding lectins, LPS-bearing proteins, C-reactive protein and serum amyloid protein. These receptors possess strong opsonic activity, and function by attaching to the microbial cell wall

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Function</th>
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<tbody>
<tr>
<td>Secreted</td>
<td></td>
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</tr>
<tr>
<td>Mannan-binding lectin</td>
<td>Terminal mannose residues</td>
<td>Lectin pathway activation of complement</td>
</tr>
<tr>
<td>Lipopolysaccharide (LPS)-binding protein</td>
<td>LPS</td>
<td>LPS recognition(^1)</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>Microbial phosphocholine</td>
<td>Opsonization, complement activation (classical pathway)</td>
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<tr>
<td>Serum amyloid protein</td>
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<td></td>
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<tr>
<td>Endocytic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophage-mannose receptor</td>
<td>Terminal mannose residues</td>
<td>Phagocytosis</td>
</tr>
<tr>
<td>Macrophage scavenger receptor</td>
<td>LPS, dsRNA, low density lipoprotein</td>
<td>Lipid homeostasis</td>
</tr>
<tr>
<td>Macrophage receptor with collagenous structures (MARCO)</td>
<td>Bacterial cell wall</td>
<td>Phagocytosis</td>
</tr>
<tr>
<td>Signaling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toll-like receptors (TLR)</td>
<td>Many microbial determinants</td>
<td>Many</td>
</tr>
<tr>
<td>dsRNA-activated protein kinase</td>
<td>dsRNA</td>
<td>Activation of NF-κB</td>
</tr>
<tr>
<td>CD14</td>
<td>LPS, peptidoglycan, monocyte, macrophage, PMN</td>
<td>Co-receptor for TLR(^1)</td>
</tr>
<tr>
<td>Nucleotide-binding oligomerization domains (NOD) receptor</td>
<td>Most NOD proteins, LPS</td>
<td>NF-κB-activated apoptosis</td>
</tr>
<tr>
<td>RP 105 (CD180, LY78, MD1)</td>
<td>Many microbial molecules</td>
<td>B cell recognition and signaling + LPS(^1) responses</td>
</tr>
<tr>
<td>MD2 (LY-96)</td>
<td>(mature B cells, dendritic cells)</td>
<td>LPS(^1) signaling response</td>
</tr>
</tbody>
</table>

\(^1\)Required for LPS recognition by TLR-4.
and priming the cells for subsequent interaction with phagocytes, complement components, and eventual cytolysis of the target cell.

Endocytic receptors are detected on the phagocyte surface, and mediate the uptake and delivery of microbial agents to the lysosomes for eventual cell lysis. The microbial proteins or peptides can also be presented by a major histocompatibility complex (MHC) molecule present on the macrophage surface. This class of PRR includes the macrophage-mannan receptor, macrophage receptor with collagenous structures, and macrophage scavenger receptors (table 1).

Signaling receptors are able to recognize a variety of PAMPs and other antigens, and activate the signal transduction pathways necessary for expression of immune response genes and a variety of cytokines [9]. The signaling PRRs include double-stranded ribonucleic acid-activated protein kinase, nucleotide-binding oligomerization domain receptors (NODs), CD14 and other receptors expressed on several host cells which bind to microbial antigens. These include RP105 and MD2 (table 1). One group of signaling PRRs which has recently attracted considerable attention is the family of toll-like receptors (TLRs).

TLRs were first isolated as a component of signaling pathways responsible for dorsoventral polarity in fly embryos [10]. The toll gene encodes a transmembrane protein with a large extracellular domain of leucine-rich repeats. The cytoplasmic domain of TLR protein is similar to the cytoplasmic domain of the mammalian interleukin-1 (IL-1) receptor. Several toll receptors have been shown to activate transcription genes of the NF-κB, the immune response genes, and other immune and inflammatory cytokine activation genes [11]. The regulation of gene expression especially for the cytokines has been shown to occur through several adapter molecules, including myeloid differentiation priming response protein 88 (MyD88), Toll/IL-1 receptor domain-containing adapter protein, mal and TLR domain containing the adapter-inducing IFN-β [12].

TLRs which are homologous to drosophila toll receptors have also been demonstrated in mammals, including man. To date, over 10 (TLR-1–10) TLRs have been identified in humans, and about 13 in murine cells. Most TLRs initiate signaling by homodimerization, although TLR-2 also forms a heterodimer with TLR-1 or TLR-6 to initiate signaling [13, 14].

Activation of the NF-κB pathway by TLR has been associated with production of such inflammatory cytokines as IL-1, IL-6, IL-8, TNF-α, IL-12, and induction of important co-stimulatory molecules such as CD80, CD86, and CD40. Furthermore adapter molecules, including MyD88, have been shown to trigger apoptosis through caspase cascade.

The specific microbial ligands associated with and the functions observed for different members of the TLR family are summarized in table 2. The expression of TLR on human cells is widespread. All TLRs, except possibly TLR-3, are uniformly expressed on monocytes and macrophages. Myeloid DCs have been shown to express TLR-1, 2, 4, 5, 7 and 8, and plasmacytoid
DCs and B cells selectively express TLR-3, 7, 9 and possibly 10. TLR-2, 4, 6 and 8 are frequently associated with mast cells. Recent studies have also suggested the expression of TLRs in epithelial cells on different mucosal surfaces [15, 16].

Although the precise role of TLRs in innate and adaptive immune functions is still evolving, it is clear that TLR activation is associated with increased antimicrobial activity, apoptosis of phagocytic cells, and increased expression of co-stimulatory molecules necessary for the induction of proinflammatory or immunoregulatory cellular functions.

The possible role of TLRs in the mechanisms of protection against or the pathogenesis and outcome of infectious diseases is currently under careful investigation. The precise contribution of different TLRs in the development of immunologically mediated disorders remains to be determined. However,
many TLRs have been linked to the development of such disease states as arteriosclerosis (TLR-1/2, TLR-4), allergy (TLR-4), HIV infection (TLR-2), IL-1 receptor kinase 4, deficiency involving signaling for several TLRs (TLR-2/1, 2/6, 5, 7, 8, 9), defects in NF-κB essential modulation associated with incontinentia pigmenti in females and 1-κB defects associated with partial blockage of NF-κB-signaling processes. Furthermore, many single nucleotide polymorphisms have been identified in some TLR genes. Polymorphism in CD14 and TLR-2 has been associated with the severity of atopic disease. However, other studies on the association of TLRs with various disease states have provided conflicting results [12, 17, 18].

**Cellular Components of Innate Immunity: Dendritic Cells**

In addition to the interaction with PRRs, a major signal expressed after exposure to microbial pathogens by the innate immune system includes the activation of the adaptive immune system, mostly via the regulation of the function of antigen-presenting cells. It has been shown that CD80 and CD86 molecules on the surface of antigen-presenting cells represent vital co-stimulatory molecules. These molecules in association with the MHC-peptide complex are essential for T cell activation, and T cell interaction with an antigen in the absence of CD80 or CD86 leads to apoptosis or inactivation of the cells. The induction of CD80 and CD86 molecules on antigen-presenting cells is controlled by the TLRs when exposed to PAMPs on specific pathogens [19]. However, it is important to note that TLR induces expression of CD80 and CD86 only after natural or acquired infections.

The DCs represent the key antigen-presenting cell and serve as a major link between innate and adaptive immune responses. As pointed out earlier, these cells recognize antigens by specific expression of PRRs that bind to different PAMPs. After antigen uptake, the DCs mature into different subsets with distinct biologic functions. Lymphoid DCs (CD8+) are often tolerogenic and myeloid DCs (CD38+) are by and large immunogenic. Mucosal DCs have now emerged as critical cells which are very important in regulating immunity to pathogens, development of mucosal inflammation and disease, and induction of mucosal (oral) tolerance. Aggregates of DCs are widely distributed in different mucosal surfaces, including lymphoid follicles, Peyer's patches, regional lymph nodes, cryptopatches, and the entire lamina propria of the small and large intestine. The lamina propria DCs are characterized by the expression of CX3 CR1 (receptor for fractalkine), CD83, CD11, and possibly other cell differentiation markers, depending on the level of functional maturity. The DC subsets can be defined by the expression of different TLRs. Isolated human plasmacytoid DCs express TLR-7 and TLR-9, whereas myeloid DCs express TLR-1, 2, 3, 4, 6, 8 [20, 21].

Mucosal DCs continually sample the environmental macromolecules, microbial antigens and other dietary antigens. Lamina propria DCs prevent dissemination of antigens to deeper tissues, and instead transport them to the
regional (mesenteric) lymph nodes to induce specific mucosal IgA response by the B cells. In addition to their activation by PAMP–PRR interactions, maturation of DCs may also be driven by stimulation via proinflammatory mediators such as TNF-α, IL-1, interferons, which are frequently released after bacterial or viral infections.

Mucosal DCs have also been shown to express c-type lectins and mannose receptors as PRRs, in addition to the TLRs. It has been shown that DCs express nucleotide-binding oligomerization domains (NOD-1, NOD-2) during antigen processing. These peptides recognize muramyl tripeptide (NOD-1), from gram-negative organisms and muramyl dipeptides (NOD-2) common to all peptidoglycans of bacterial species [22].

Based on the information obtained to date, DCs in mucosal surfaces may have several functions. The major defenses appear to be related to the production of IL-12, and type I interferons which influence the polarization of T helper cells (Th1 CD4+), the development of cytotoxic T cells, induction of antibody responses and memory cells, or the development of tolerance. The DCs possess remarkable ability for dendrite formation and antigen sampling, and migration to defined sites, in particular the T cell sites in the lymphoid organs, and sites such as skin, lung, solid organs and different areas of mucosal epithelium and lamina propria. The migration process is significantly influenced by bacterial LPS and other PAMPs, TNF-α, IL-1, and by specific microbial parasitic, or viral, agents [23].

It appears that antigen recognition in the mucosal immune system is obligatory for induction of tolerance and, depends on CCR7-mediated cell migration. More specifically, antigen presentation by lamina propria DCs appears to be critical for induction of oral tolerance. On the other hand, it has also been demonstrated that dysregulated recognition of intestinal microflora by DCs may be a major factor for the induction of mucosal disease, such as inflammatory bowel disease in genetically susceptible individuals. Mutations of NOD-2 have been associated with Crohn’s disease. More recently, animal experiments have suggested that intestinal DC function can be significantly altered by certain enteric pathogens. DC activation by *Heligmosomoides polygyrus* and expression of IL-10 was found to impair host protection against *Citrobacter rodentium* infection and the development of severe mucosal injury [24]. Thus, mucosal DCs may be the sentinel cellular element responsible for the outcome of antigenic exposure in innate as well as adaptive immune responses, on the mucosal surfaces.

**Adaptive Immunity**

In contrast to the innate immune system, the receptor repertoire of T and B lymphocytes is generated somatically during their development. Significantly, however, since they are not encoded in the germ line, these receptors are not
predetermined for recognition of any specific pathogens, PAMPs, or antigens. A diverse receptor repertoire is generated randomly during development. However, certain lymphoid cell populations bearing receptors for selected pathogens or other antigens are selected for clonal expansion after antigenic exposure. Such receptors are not transmitted to the next generation and as a result have to be regenerated or re-invented for every newborn infant of the species.

Because of the random nature of T and B cell repertoire development, the immune response can, under certain circumstances, be directed against otherwise self (autoantigens, neoantigens), or other benign environmental agents, resulting in the development of autoimmune or other immunologically mediated disease processes.

The ability of the host to discriminate and selectively modulate the immune response (immune response vs. tolerance) to self or environmental antigens appears to be largely a function of the mechanisms of innate and adaptive immunity operating on external mucosal surfaces, the primary port of entry for most microbial pathogens and dietary antigens in the mammalian host.

**Components of Mucosal Immunity**

In addition to the defined elements of innate immunity described above, mammalian mucosal surfaces possess several nonspecific, but highly effective mechanisms of defense and local repair. These include trefoil peptides produced by goblet cells. The trefoil factors also play an important role in protection against bacterial toxins and effect intestinal epithelial repair following injury. Another important product is defensin generated by the Paneth cells, specialized epithelial cells derived from the intestinal stem cells. Paneth cells secrete antimicrobial lysozymes and phospholipase A2. These cells preferentially disrupt microbial cell membranes and effect cell death [2].

The organized lymphoid follicles in the intestine (gut-associated lymphoid tissue) and bronchial subepithelial regions, and nasopharyngeal tonsils are considered to be the principal inductive sites of mucosal immune responses. Under certain circumstances, the appendix, peritoneal precursor lymphoid cells and rectal lymphoepithelial tissue (rectal tonsils) may also serve as inductive sites of local immune responses [2].

Recently, the crypt lamina propria of the mouse small intestine has been shown to harbor tiny lymphoid clusters endowed with cells positive for IL-7 receptor. These clusters have been referred to as cryptopatches. The lymphoid structures may represent yet another important inductive element of gut-associated lymphoid tissue [24].

The development of Peyer's patches and other follicle-associated lymphoepithelium is first observed around 10–11 weeks of gestation (table 3). It has been demonstrated that during fetal growth, progenitor lymphoid tissue-inducer cells populate the developing lymph nodes and Peyer's patches. In
the adult, similar lymphoid tissue inducer-like cells support the formation of gut-associated lymphoid tissue, cryptopatches and other isolated lymphoid follicles. Interestingly, these cells are also located in close proximity to mucosal DCs [25–29].

The common features of all inductive mucosal sites include an epithelial surface containing M cells overlying organized lymphoid follicles. Their ultrastructural and functional characteristics were extensively defined in the early 1970s. The mucosal epithelium is a unique structure and in addition to M cells, it contains mucin-producing glandular cells, lymphocytes and plasma cells, DCs and macrophages. The mucosal epithelial cells express polymeric immunoglobulin receptor (PigR) and secretory component (SC), MHC class I and II molecules, other adhesion molecules, and a variety of cytokines and chemokines.

The M cells are important in luminal uptake, transport, processing and to a smaller extent in the presentation of mucosally introduced antigens. The M cells appear to be critical in the transport, and entry of organisms such as reovirus, poliovirus, rotavirus and salmonella into the human host. M cell-mediated antigen uptake is characteristically associated with the development of secretory IgA (S-IgA) and other mucosal specific responses [2].

The luminal appearance of S-IgA in mucosal secretions results from transcytosis of polymeric IgA (pIgA) across the mucosal epithelium via binding to PigR. The receptor is eventually cleaved resulting in the association of pIgA with a substantial part of PigR. The complex of IgA and PigR is generally referred to as S-IgA.

**Table 3.** Development of Peyer’s patches in human neonate

<table>
<thead>
<tr>
<th>Period of life</th>
<th>Age</th>
<th>Features of Peyer’s patch lymphoid tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenatal</td>
<td>10–11 weeks</td>
<td>Rudimentary patches</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HLA-DR+CD4+ cells</td>
</tr>
<tr>
<td></td>
<td>11–16 weeks</td>
<td>CD8+ cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface IgM, IgD+B cells</td>
</tr>
<tr>
<td></td>
<td>16–18 weeks</td>
<td>CD5+B cell</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgA+ B cells</td>
</tr>
<tr>
<td></td>
<td>18–20 weeks</td>
<td>Appearance of B and T cell zones</td>
</tr>
<tr>
<td></td>
<td>24 weeks</td>
<td>Visible Peyer’s patches</td>
</tr>
<tr>
<td>Postnatal</td>
<td>24 h to 6 weeks</td>
<td>Formation of germinal centers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>after mucosal antigen exposure</td>
</tr>
<tr>
<td>Average number of patches</td>
<td>24 weeks gestation</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Birth and perinatal period</td>
<td>305</td>
</tr>
<tr>
<td></td>
<td>12–14 years</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>20 years</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>90 years</td>
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</table>
Following exposure to an antigen and its uptake via the M cells, there is a variable degree of activation of T cells, DCs, and B cells especially of the IgA isotype. The interaction of lymphocytes with mucosal epithelium is important in the differentiation of some segments of the mucosal epithelium into M cells. The activation of T cells results in the release of a number of cytokines or chemokines from different T cell subsets, and recognition of plasma cells. Such differentiation involves interaction with a variety of cytokines and T cell subsets. The switch of IgM B cells to the production of IgA can also occur without T cell help.

Locally produced IgA consists mainly of J chain-containing dimers and the larger pIgA that is selectively transported through epithelial cells by the PigR. The resulting S-IgA molecules are designed to participate in immune exclusion and other immunologic functions at the mucosal surface. IgG also contributes to such surface defense. It often reaches the secretions by passive diffusion from the blood stream and, less frequently, by local synthesis. However, its proinflammatory properties render IgG antibodies of potential immunopathologic importance when IgA-mediated mucosal elimination of antigens is unsuccessful. T helper cells, activated locally mainly by a Th2 cytokine profile, promote persistent mucosal inflammation with extravasation and priming of inflammatory cells, including eosinophils. This development may be considered as a pathologic enhancement of local defenses. It appears to be part of the late phase allergic reaction, perhaps initially driven by interleukin-4 (IL-4) released from mast cells subjected to IgE-mediated or other types of degranulation, and subsequently maintained by further Th2 cell stimulation. Eosinophils are potentially tissue-damaging, particularly after priming with IL-5. Various cytokines upregulate adhesion molecules on endothelial and epithelial cells, thereby enhancing accumulation of eosinophils and, in addition, resulting in aberrant immune regulation within the epithelium. Soluble antigens available at the epithelial surfaces normally appear to induce various immunosuppressive mechanisms, but such homeostasis seems to be less potent in the airways than the induction of systemic hyporesponsiveness to dietary antigens in the gastrointestinal tract.

Numerous cytokines and chemokines have been shown to be intimately involved in the induction and maintenance of antigenic epitopes involving MHC class I or II molecules. Both Th1 and Th2 cells appear to benefit the development of S-IgA responses. Th2 cytokines (IL-4, IL-5, IL-6, IL-9, IL-10, IL-13) are thought to be of significant help in antibody production. S-IgA antibody response is also enhanced by immunologic adjuvants such as cholera toxin which results in polarized Th2 cell response. S-IgA antibody response may also be induced through Th1 cytokines (IL-2, IFN-γ) as shown with studies on intracellular pathogens such as salmonella.

It appears that the process of isotype switching of B cells to pIgA-producing plasma cells begins in mucosal inductive sites. Such switching requires specific signals by co-stimulatory molecules including cytokines.
and T helper cells. However, Th1 and Th2 type cytokines appear to contribute only minimally to the switching of B cells to surface IgA-positive B cells. Such switching is greatly enhanced by transforming growth factor (TGF)-α. Following activation and acquisition of antigen specificity, the IgA-producing cells migrate to the lamina propria of the effector sites in the mucosal tissues, regardless of the site of initial antigen exposure. There is, however, a preponderance of homing to the original site of antigenic exposure. The migration of antigen-sensitized cells is preferentially determined by the concurrent expression of integrins and homing-specific adhesion molecules in the tissue endothelium, especially mucosal addressin cell adhesion molecule-1 (MAdCAM-1) and the specific receptors (integrins) expressed on activated lymphoid cells. Oral (intestinal) mucosal exposure to antigen seems to favor expression of α4β7 integrins, and intranasal immunization has been shown to induce expression of L-selectin as well as α4β7 integrins. However, systemic immunization is generally restricted to the expression of L-selectin. This information has been reviewed in more detail elsewhere [2].

Recent studies have provided extensive characterization of the T cell in the human neonatal mucosal tissues especially in the intestine. Peyer’s patch T cells are largely CD4+, while most intraepithelial lymphocytes are CD8+, derived from CD7, CD3+ cells. The lamina propria T lymphocytes are also largely derived from CD7CD2+ cells, and over 50% of these cells are CD4+, although up to 20 and 30% of cells are CD8+ and CD4− CD8− cells, respectively [30–32] as shown in table 4 [32].

S-IgA can be detected as early as 1 week after birth and significant salivary IgA levels are detected by 4–6 weeks. However, the levels continue to rise up to 18 months of age. During the first year of life, there is also a switch from monomeric to polymeric S-IgA. At birth S-IgA subclass 1 predominates but the levels of S-IgA subclass 2 predominate by 6 months of age [33–35].

The diversity of interactions of the Fc region by the IgA molecule with specific receptors provide S-IgA with many unique functional attributes. Available evidence for the role of different IgA receptors in immunologic homeostasis is summarized in table 5. The epithelial PigR on mucosal epithelial cells transports pIgA to the mucosal surface, where in complex with the SC, immunoglobulin A (S-IgA) contributes to the exclusion of the multitude of dietary, environmental, and microbial antigens. IgA-mediated exclusion forms a part of the initial defense against infection. It also spares the systemic immune system from potentially deleterious responses to innocuous antigens which can otherwise culminate in disease. Other IgA receptors may contribute to protective immunity and prevention of disease. FcaRI is the principal myeloid IgA receptor and is responsible for effector responses such as respiratory burst, degranulation, and phagocytosis by granulocytes, monocytes, and macrophages. Furthermore, an IgA receptor specific for the SC elicits powerful effector responses from eosinophils. On DCs, FcaRI participates
in antigen presentation, while on M cells another IgA receptor may function in the transport of antigens across the mucosal epithelial barrier. The expression of a still to be characterized IgA1/IgD receptor on T cells may affect the development of autoimmune disorders. The interplay of several different IgA receptors has been shown to affect immune complex deposition in IgA nephropathy [36].
Regulation of Mucosal Immune Responses

The outcome of host–pathogen–other environmental macromolecular interactions are determined by the elaboration of many potent cellular and soluble products in the host. These include proinflammatory or immunoregulatory cytokines or chemokines, specific antibodies, the development of various T cell activation processes, and natural killer (NK) cell expression. Such responses, either alone or in well-orchestrated mechanisms, cause destruction of specific microorganisms, or infected cells, tumors or autoreactive cells. An important byproduct of such events may be the expression of severe injury or death of host cells as well. As a result, a complex process of regulation of the immune response has evolved. This includes, NK cells (NKT), naturally occurring CD25+/CD4+, inducible (adaptive) CD5+/CD4+ T helper cells, CD8+ T suppressor cells, T cell receptor (TCR)-specific anti-idiotypic cells, and anti-ergotypic TCR-nonspecific T cell populations. The CD25+/CD4+ T cells are apparent by 13–14 weeks of gestation in the human fetus and represent about 5–10% of CD4+ T cells at birth, a proportion similar and slightly more frequent than in the adults [37–39]. Considerable attention has focused on four subpopulations of regulatory T cells, CD4+ helper T cells (Th1, Th2, Th3), and Th17/ThIL-17 cell population [40, 41].

Although the immunologic repertoire for regulatory functions in the human neonate is competent and responds effectively to microbial pathogens and other environmental antigens, certain differences are characteristic of neonatal T cell function. These include, a higher overall T cell number including CD4 and CD8+ cells than observed in the adult. Most neonatal T cells are naive in phenotype and function, over 90% of the cells are CDRA45+. Most neonatal T cells also possess high activation threshold and co-stimulation dependence for IL-2 production. Neonates exhibit lower production of IL-4 and IFN-γ, and impaired initial expression of CD40. However, these parameters return to normal adult values after activation-induced proliferation [42].

There are also subtle but significant differences in antigen-specific T cell responses in the neonatal period [42]. These include delayed skin reactivity to Mycobacterium tuberculosis, delayed appearance of CD4+ response after perinatal infections with herpes simplex virus or cytomegalovirus. The T cell-independent B cell antibody responses are generally absent at birth and fully mature T cell-independent B cell responses occur by 3–5 years of age. On the other hand, a T cell-dependent B cell response can be detected shortly after birth to most protein antigens (table 6). Detailed information about neonatal immune responses is provided in other presentations given here. It is, however, important to note that the delayed maturation of T cell function in the neonatal period preferentially appears to affect Th1 responses. The precise mechanisms underlying this physiologic delay remains to be determined. It has been proposed that deficient induction of co-stimulatory molecules and Th1/inducer cytokines, alone or in concert with intrinsic T cell dysfunction may be largely responsible for these observations [42].
It has been proposed that Th1 maturational delays may be genetically determined and significantly influenced by the environment. Temporal studies on neonatal immunocompetence carried out by Holt et al. [43–45] have suggested that non-atopic infants will under normal physiologic conditions exhibit increasing Th1 response as the child grows. However, atopic subjects conspicuously fail to exhibit such improvements in the Th1 response. Conversely, it appears that over time the Th2 responses decrease in normal non-atopic subjects, but the atopic subjects will continue to exhibit increasing Th2 responses [43–45].

**External Environment: Impact on Immunologic Homeostasis**

**Geophagy: Eating Dirt**

The relationship between the external environment and human health and disease must be as old as the evolutionary biology of mammalian life itself. In a fascinating report, Callahan [46] describes an interesting ritual of eating dirt from the earth at the conclusion of daily church services in a shrine in Esquipales, Guatemala; as well as in the Chapel of El Santuario de chomayo in the hills of Northern New Mexico.

Eating dirt is a common practice in virtually every mammalian species including primates [47]. What is, however, amazing is that geophagy is also
intentionally practiced by many human groups at different ages and in differ-
ent parts of the world. Geophagy is considered normal, without any major
adverse and sometimes even with some beneficial effects in most animals. On
the other hand, this behavior is considered by many to be abnormal for
humans, and referred to as soil pica. It has been reported that non-pathologic
dirt eating is not an uncommon practice by pregnant women in sub-Saharan
Africa, migrants from this culture in other parts of the world and by young
children during their formative years worldwide. From a contemporary
behavioral standpoint, it has been proposed that consumption of >50 g of
soil/day should be considered a pathologic soil pica [46].

In today’s human society, soils contaminated by sewage, animal wastes,
industrial or human pollutants, poses considerable risk from infections, tox-
ins, heavy metal poisoning and carcinogens. It should be taken into account
that the type of soil unaffected by human waste, and other man-made envi-
ronmental products enjoyed by our ancestors must have been quite different
than the top crust of earth’s soil today.

Since recorded history, it has been almost impossible to keep children
away from dirt. The EPA estimates suggest that children in the USA consume
on an average 200–800 mg of dirt/day and some children may in fact consume
more [46]. Most common infections associated with soil pica are *Toxocara
canis*, raccoon round worm, and ascaris infection [48]. However, all parasitic
agents that infest soil do not uniformly infect humans who eat dirt, nor do all
subjects who eat dirt contract disease routinely [46]. It has been estimated
that there are about 4,600 species of prokaryotic microorganisms per gram of
natural soil, and about 7,000 g of biomass per cubic meter of soil [49, 50].

*Biomass and Microbial Flora of Mucosal Surfaces*

Since the evolution of non-nucleated single cell life forms over 4 billion
years ago, most microorganisms, especially the bacteria, have continued to
develop a complex relationship with other life forms including man and other
mammals. It has been estimated that more than $10^{29}$ bacteria live on the
planet and as many as $10^{14}$ (or over 100 trillion), comprised of over 500
species of microorganisms live in or on each human being [46, 51]. This rela-
tionship begins shortly after birth and continues throughout a person’s life
span. At the same time, it is estimated that a fully developed human contains
about 10 trillion ‘human’ cells derived from the original fertilized egg. Thus,
we must accept the sobering fact that for every human cell, there may be as
many as 10 or more bacterial cells living in constant symbiosis with the
human cells in the human body [52]. In the human gut alone, the total weight
of microflora is estimated to be 1 kg. Thus, the collective genome of our colo-
nizing bacterial commensals must be incorporated in the comprehensive view
of health and disease of all human and other mammalian life forms.

Microbial colonization of the human mucosal surface begins at birth after
exposure to maternal microbial flora in the genital tract and gut. The neonate
is initially colonized by enterobacteria and may reach a microbial concentration of as much as $10^9/g$ of feces. By the beginning of the 2nd week of life bifidobacteria predominates in the breastfed infant, whereas enteric organisms remain the predominant organism in formula-fed infants [53, 54]. By 1 month of age, bifidobacteria predominates in both groups, although their proportion is about 10 times higher in breastfed infants. Furthermore formula-fed infants exhibit a more complex microflora with bacteroides, clostridia and streptococcal species contributing significantly to the resident population of organisms. The significance of the differences in the composition of mucosal microflora between breastfed and formula-fed infants are not fully understood. However, the rich diversity of immunologic products in the maternal milk, type of proteins ingested, availability of iron, presence of oligosaccharides and other products in the breastfeeds appear to play a very important role in determining the nature of microbial colonization and subsequent immune responses to environmental and dietary antigens [55].

**Bacterial Flora and Outcome of Immune Response**

There is now sufficient evidence to suggest that mucosal microflora directly influences the outcome of mucosal immune responses. Nonpathogenic salmonella infections in in vitro (tissue culture) settings have been shown to inhibit the development of mucosal inflammation by blocking NF-κB-induced activation of genes coding for inflammatory cytokine expression [56]. Similarly colonization by commensals such as thetaiotaomicron (a bacteroides species) in germ-free mice induces decay-accelerating factor. The decay-accelerating factor appears to inhibit cytotoxic damage from microbial activation by complement components, C-reactive protein, ductin, (a possible receptor for intestinal mucosal trefoil factors) and sprrza, a family of proline-rich proteins involved in barrier functions [57]. Finally there is strong evidence to suggest that the nature of commensal bacterial flora acquired during the neonatal and early postnatal period is necessary for the development of tolerance to dietary proteins. Development of tolerance to IgE production against ovalbumin in the intestine was found to require colonization with a single, or polymicrobial flora in the intestinal mucosa [58].

Microbial colonization also appears to affect expression of host genes that regulate postnatal maturation, nutrient uptake and metabolism, processing of xenobiotics and development of angiogenesis [57]. Increased expression of genes regulating carbohydrate absorption may also explain the increased need for increased caloric consumption (by over 30%) in germ-free mice to sustain body weight similar to conventional mice [58]. It is possible that similar differences in microbial flora in early infancy could affect the nutritional outcome in obese vs. lean individuals in later life. The nature of microbial colonization in different segments of human mucosal surfaces reflects very unique patterns. Studies reported by Alderberth et al. [59] have suggested that the microbial load in different body surfaces ranges from essentially no organisms in the
normal lung, to <10³/ml in the stomach, about 10³⁻⁵/ml in small intestine, 10¹⁰⁻¹¹/ml in large intestine (feces), and about 10⁸⁻¹⁰/ml in the nasopharynx and the lower urinary tract and the female genital tract. It appears that both microbial, as well as host-derived factors facilitate residence of different segments of human mucosal surfaces. These include inter- and intra-species communication and quorum sensing, and biofilm formations. Examples of such sophisticated arrangements between the host and microbial flora exist in other settings, such as dental plaques where the biofilm-commensal plaque is assembled through a complex process of adhesion of early arriving anaerobic streptococcal species to the dental pellicle (host-derived) on the surface of the tooth. This is subsequently followed by secondary interbacterial adhesion and intergenic communications with other organisms, with further bacterial adhesions, resulting in the formation of a distinct bacterial structure of organisms on a defined tissue site [60, 61]. In more recent studies carried out in infants delivered via cesarean section or after normal vaginal delivery, infants delivered by cesarean section were found over time to be colonized more often with klebsiella and enterobacter than infants delivered vaginally. Whereas bacteroides species colonized about 30% of vaginally delivered infants and the colonization rate increased slowly, significant colonization by such anaerobes was delayed by up to 1 year in cesarean section deliveries [62].

**Immunologic Outcome of Host–Microbial Interventions**

The information summarized in the preceding sections has established a framework for the impact of microbial flora and other macromolecules in the environment on host immunologic homeostasis, and of the host, on the microbial flora continuously entering the host's mucosal surfaces.

The potential impact of environmental factors on the development of T cell responses and disease association is summarized in table 7. In addition to these observations, it is felt that the prevalence of several possibly immunologically mediated diseases has drastically increased over the past century in most regions of the world as the socioeconomic conditions have changed [63, 64]. At the same time, it is felt that many tropical infectious diseases continue to benefit the host by their ability to confer protection against certain immunologically mediated diseases, while other infections may adversely contribute to the development of other allergic or immunologically mediated disorders.

**Allergic Disorders: Protective Effects of Microflora**

Certain environmental antigens, dietary proteins and other external or endogenous allergens are commonly associated with the development of such allergen-specific diseases as asthma, hay fever, eczema, anaphylactic shock, or allergic rhinitis. Such disease processes are thought to be initiated by the CD4⁺ Th2 lymphocyte subpopulation. These cells are responsible for producing
IL-4, IL-5, IL-9, and IL-13. Th2 cytokines are also responsible for the regulation of IgE and other immunoglobulin-isotype production by B cell development and recruitment of eosinophils and other inflammatory cells, contractility of airway smooth muscle, and mucous production. Earlier epidemiologic studies and more recent in vivo and in vitro experiments have suggested that some infections may provide significant protection against the development of allergic diseases, despite the ubiquitous nature of environmental allergens (table 8). For example, the presence of a positive tuberculin skin test prior neonatal immunization with bacillus Calmette-Guérin (BCG) vaccine and/or infection with tuberculosis before 20 years of age are significant markers of protection against the development of allergic disorders later in life [64–67]. The anti-allergic effects of mycobacteria may be related to modulation of Th1 responses and by other Th1-independent mechanisms, involving IL-10, TGF-β and induction of CD11c cells [68].

In addition to mycobacteria, infections with cell-associated pathogens such as Chlamydia trachomatis, Listeria monocytogenes, and intradermal inoculation of BCG have been associated with significant improvement in the clinical scores of atopic dermatitis, but not the severity of asthma [69]. However, in one other study, replicating live BCG vaccine was found to result in a significant improvement in asthma scores in adults [70].

Table 7. Evolution of cytokine paradigms in the mucosal surfaces and their possible disease association

<table>
<thead>
<tr>
<th>Increased Th1 cytokine profile</th>
<th>Increased Th2 cytokine profile</th>
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<tbody>
<tr>
<td>Environmental factors</td>
<td></td>
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<tr>
<td>Normal commensal flora</td>
<td>Diet in developed world</td>
</tr>
<tr>
<td>Inflammatory cytokines</td>
<td>Processed foods</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>Introduction of cow’s milk and formula products</td>
</tr>
<tr>
<td>Many infections¹</td>
<td>Antibiotic use</td>
</tr>
<tr>
<td></td>
<td>Infections</td>
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<tr>
<td>Disease association</td>
<td></td>
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<tr>
<td>Autoimmune thyroiditis</td>
<td>Leishmaniasis</td>
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<tr>
<td>Experimental autoimmune</td>
<td>Mycobacterial infection (M. TB, M. leprae)</td>
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<tr>
<td>uveo-retinitis</td>
<td></td>
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<tr>
<td>Crohn’s disease</td>
<td>Candida</td>
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<tr>
<td>Other immunologically</td>
<td>Toxoplasmosis</td>
</tr>
<tr>
<td>mediated disorders (EAE,</td>
<td>HIV</td>
</tr>
<tr>
<td>MS, IDDM)</td>
<td>Asthma</td>
</tr>
<tr>
<td></td>
<td>Atopic dermatitis</td>
</tr>
<tr>
<td></td>
<td>Allergic rhinitis</td>
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</tbody>
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EAE = Experimental allergic encephalitis; HIV = human immune deficiency virus; MS = multiple sclerosis, IDDM = insulin-dependent diabetes mellitus.
¹Also see table 8.

IL-4, IL-5, IL-9, and IL-13. Th2 cytokines are also responsible for the regulation of IgE and other immunoglobulin-isotype production by B cell development and recruitment of eosinophils and other inflammatory cells, contractility of airway smooth muscle, and mucous production. Earlier epidemiologic studies and more recent in vivo and in vitro experiments have suggested that some infections may provide significant protection against the development of allergic diseases, despite the ubiquitous nature of environmental allergens (table 8). For example, the presence of a positive tuberculin skin test prior neonatal immunization with bacillus Calmette-Guérin (BCG) vaccine and/or infection with tuberculosis before 20 years of age are significant markers of protection against the development of allergic disorders later in life [64–67]. The anti-allergic effects of mycobacteria may be related to modulation of Th1 responses and by other Th1-independent mechanisms, involving IL-10, TGF-β and induction of CD11c cells [68].

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Considerable investigative effort with probiotics has generated interesting information about their usefulness in the control of allergic diseases. Studies of lactobacillus, bifidobacteria and other probiotics have suggested a strong correlation between maternal (prenatal use) or the use of infant-formula.

### Table 8. Possible role of different infections in the mechanism of protection against or pathogenesis of allergic and autoimmune disorders

<table>
<thead>
<tr>
<th>Microbial agents</th>
<th>Clinical disorders and role of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>allergic</td>
</tr>
<tr>
<td></td>
<td>protective</td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
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<tr>
<td>Bordetella</td>
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<tr>
<td>Borrelia (Lyme disease)</td>
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<tr>
<td>Chlamydia</td>
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<tr>
<td>Conventional microflora</td>
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<tr>
<td>Pathogen-free flora</td>
<td></td>
</tr>
<tr>
<td>Lactobacilli, other probiotics</td>
<td>+</td>
</tr>
<tr>
<td>Mycobacteria, BCG</td>
<td>+</td>
</tr>
<tr>
<td>Salmonella</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td></td>
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<tr>
<td>Streptococcus group A</td>
<td></td>
</tr>
<tr>
<td>Parasites</td>
<td></td>
</tr>
<tr>
<td>Ascaris</td>
<td>+</td>
</tr>
<tr>
<td>Fasciola</td>
<td>+</td>
</tr>
<tr>
<td>Filaria</td>
<td></td>
</tr>
<tr>
<td>Heligmosomoides</td>
<td>+</td>
</tr>
<tr>
<td>Hookworm</td>
<td>+</td>
</tr>
<tr>
<td>Hymenolepis</td>
<td></td>
</tr>
<tr>
<td>Nippostrongyloides</td>
<td>+</td>
</tr>
<tr>
<td>Schistostoma</td>
<td></td>
</tr>
<tr>
<td>Strongyloides</td>
<td>+</td>
</tr>
<tr>
<td>Toxocara</td>
<td>+</td>
</tr>
<tr>
<td>Tricuris</td>
<td></td>
</tr>
<tr>
<td>Viruses</td>
<td></td>
</tr>
<tr>
<td>Coxsackie</td>
<td></td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>+</td>
</tr>
<tr>
<td>Influenza</td>
<td>+</td>
</tr>
<tr>
<td>Measles</td>
<td></td>
</tr>
<tr>
<td>Metapneumovirus</td>
<td>+</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>+</td>
</tr>
<tr>
<td>RSV</td>
<td>+</td>
</tr>
<tr>
<td>Rubella</td>
<td></td>
</tr>
<tr>
<td>Theiler's</td>
<td></td>
</tr>
</tbody>
</table>

+ = Positive association.
containing *Lactobacillus rhamnosus* or *Bifidobacterium lactis* in the neonate and subsequent protection against atopic dermatitis [71]. However, such dietary intervention had little or no benefit in patients with established asthma or food allergies [72].

Infestations with common parasites in the tropics have also been associated with decreased incidence of allergic diseases. Infestations with hookworm, ascaris, toxicara, and schistosomes have been associated with reduced expression of atopy and of the atopic phenotypes [73, 74]. Other studies have shown that specific antiparasitic chemotherapy instituted for long-term intestinal helminths was associated with a significant increase in mite skin test reactivity in allergic subjects [75]. Recently, in vivo experimental studies in mice have shown that introduction of *Nippostrongylus brasiliensis* and *Strongyloides stercoralis* result in suppression of allergic responses in the lungs and a decline in allergen-induced eosinophilia and eotaxin production. These beneficial effects of parasitic infestations appear to be IL-10-dependent [76–79]. In addition to the bacterial and parasitic infestations listed above, epidemiologic observations have also suggested a protective role of viruses such as hepatitis A virus, respiratory syncytial virus (RSV) G protein and influenza A virus on the development of allergic diseases [80–83].

There is also evidence to suggest a strong role for bacterial–viral interactions in human mucosal surfaces, on the outcome of certain viral-induced inflammatory disease processes. Several investigators have observed protective effects of bifidobacteria, *B. bifidum* and lactobacilli on the course of murine and human rotavirus infection, gut endotoxin concentration, and human intestinal barrier functions [84–86]. Experimentally induced mucosal viral infections have also been shown to alter mucosal permeability and subsequent IgE responses to dietary antigen and other environmental allergens [87–89].

**Allergic Disorders: Pathogenic Effects of Microflora**

Although most infection processes in the tropical setting have been found to be protective against allergic diseases as outlined above, there is evidence to suggest that some infections may in fact facilitate or directly induce allergic disease manifestation (table 8). RSV, influenza, rhinoviruses and the recently identified metapneumovirus appear to be risk factors for the development for childhood asthma [90, 91]. Additional evidence has suggested that chlamydia and mycoplasma may also contribute to the development of childhood asthma. In other studies, *Staphylococcus aureus* and *Bordetella pertussis* have been associated with increased expression of allergic disorders by enhancement of inflammation of the skin and airways, respectively [92–95]. Many helminths have been implicated in the development of allergic disorders. These include natural or experimentally induced infestation with ascaris and toxicara [74], *Fasciola hepatica* [96], *N. brasiliensis* [74], and *Strongyloides venezuelensis* [97] (table 8).
Autoimmune Disorders: Protective Effects of Microflora

Unlike allergic diseases, autoimmune disorders are thought to result from tissue damage secondary to an abnormal immune response to infectious or infection-induced neoantigens in the host. As outlined in table 7, some infections seem to provide protection against the development of autoimmune diseases, while others may in fact contribute to their evolution in the human host and in animal models. It has been suggested that exposure to mycobacteria, salmonella, certain viruses and several helminths provide significant protection against the development of insulin-dependent diabetes mellitus in experimental murine models [63, 98–100]. Clinical studies have raised the possibility that certain infections within the first few years of life, decrease the risk of developing inflammatory bowel disease, multiple sclerosis and possibly insulin-dependent diabetes mellitus [101, 102]. Experimental studies with murine models have also provided some evidence of protection afforded by other parasitic agents. These include schistosomes in diabetes in non-obese diabetic mice [103], lactobacilli, or *Heligmosomoides polygyrus* in spontaneous colitis, or inflammatory bowel disease [104–106], *Trichuris suis* and filarial antigen E562 in Crohn's disease [107], and other autoimmune disorders [108].

Autoimmune Disorders: Pathogenic Effects of Microflora

While many parasitic infestations and several bacterial infections have been associated with varying degrees of protection against autoimmune diseases discussed above (table 8), it is interesting that some of the classic examples of autoimmune disease are those which occur shortly after certain infectious insults. These include post-infectious acute rheumatic fever, encephalitis, or glomerulonephritis after group A hemolytic streptococcal infection; infection with antibiotic-resistant Lyme arthritis; congenital rubella virus infection and development of insulin-dependent diabetes mellitus (IDDM), and chronic myocardial disease or IDDM after Coxsackie B virus infections [108–111]. Spontaneous development of experimental allergic encephalomyelitis is not seen in mice maintained in pathogen-free microflora, but is characteristic in mice kept with conventional microbial flora [108]. There is also evidence to suggest that direct intracerebral inoculation can result in the development of autoreactive T cells followed by the development of demyelinating encephalitis [112].

Microbial Mechanisms of Disease Production or Protection

Allergy

It is clear that microbial exposure under natural as well as controlled experimental situations can protect or reduce the risk of disease, or induce or
aggravate the clinical expression of allergic and autoimmune disease processes. Although the precise mechanism underlying the protective or pathogenic roles of different infections in these two disease states remains to be defined, several explanatory mechanisms have been proposed.

Currently, it is believed that increased Th2 CD4+ T cell responses to dietary proteins, environmental allergens, and other external or endogenous antigens, provoke the development of allergic diseases. Th2 CD4+ cells produce a number of cytokines and chemokines, including IL-4, IL-5, IL-9, IL-10, IL-13, and granulocyte-macrophage colony-stimulating factor, after exposure to specific peptides of different allergens presented by mucosal antigen-presenting cells. In addition, Th2 cytokines induce activation of B cells responsible for IgE, IgA and other isotype of antibody response. Such allergen-specific cellular products and antibody responses lead to many immunological events, including the recruitment and activation of eosinophils and mast cells and their degranulation, mucous production, and development of smooth muscle hyperactivity. Such Th2 effects are augmented by Th1 CD4+ T cell responses that lead to chronic inflammation. Other mechanisms involved in the development of allergic disorders appear to be associated with increased activation of IL-8, which favors recruitment of polymorphonuclear leukocytes [113] and IL-17, a potent stimulus for mucus production [114]. Other products which have been implicated in the development of allergic diseases include other chemokines, such as IL-13 [115], and other immunoregulatory cytokines [116].

The beneficial effects of infections on the outcome of allergic disease may be related to the following immunoregulatory events. (a) Increased induction of Th1 CD4+ T cell responses associated with increased IFN-γ production. Such responses have been shown to inhibit the Th2 response after infection with mycobacteria, influenza and RSV. (b) Increased production of TGF and IL-10 cytokines. Studies with certain mycobacterial species have shown that allergen-induced bronchial hyperactivity is significantly inhibited by increased IL-10 production and not by IFN-γ-producing Th1 cells [65, 117]. Similar mechanisms may exist for the effects of helminthic infestations and protection against allergy [78, 79]. (c) There is experimental evidence to suggest that certain mycobacterial infections induce the selective development of CD11c cell populations. These cells produce IL-10 and TGF-β [68].

Autoimmunity

Expression of autoimmune disorders as pointed out earlier is considered to be a reflection of immunologic response by the host to self (auto or neo) antigens, resulting in abnormal cytokine production and subsequent recruitment and activation of effector cell mechanisms and specific tissue damage. The development of infection-induced altered tissue antigens (neoantigens), exposure of otherwise hidden tissue antigens and the appearance of autoreactive T and B cells are normally seen but to a lesser extent in a healthy subject’s immunologic repertoire. As mentioned earlier, infections with
mycobacteria, salmonella, several helminthic agents, and possible viral hepatitis A are strongly associated with decreased evidence of autoimmune diseases, especially for the occurrence of IDDM inflammatory bowel disease, and encephalitis. The possible mechanisms which mediate infection-induced protection against autoimmunity include the following. (a) Infections induce IL-10 and TGF-β. These cytokines may directly inhibit expression of autoimmunity. IL-10 production may also induce selective activation of Treg lymphocytes which have been shown to suppress autoimmune responses by inhibiting autoreactive T cell function largely by altering cell-to-cell contact, and by IL-10 and TGF-β production. (b) Helminthic and mycobacterial infections can induce activation of NK cells (NKT). Such cells induce cytotoxicity for autoreactive antigen-bearing cells and can thus inhibit the evolution of autoimmunity [103]. (c) Certain infectious agents, especially probiotic bacteria induce Th1 CD4+ responses and such a response can directly lead to reduced expression of autoimmunity. (d) Recently, it has also been suggested that a population of CD25+ T cells characterized by the expression of Foxp3 transcription factor may also be important in mediating protection against inflammatory bowel disease, independent of IL-10 production [105].

As pointed out earlier, while most helminthic and several bacterial infections appear to be protective against autoimmunity, many viral infections and some bacterial pathogens have been highly associated with the development of autoimmune disorders. Several explanations have been proposed to explain this association. (a) Direct cytolytic effects of some bacterial, viral and parasitic infection may lead to increased expression of infection-induced cellular self (autoreactive) neoantigens, or tissue determinants otherwise hidden in a normal setting. Such cytolytic processes can lead to increased presentation of autoantigens by DCs, macrophages and other antigen-presenting cells, resulting in autoreactive Th1, Th2, CD8 + CTL, NKT and/or B cell responses with subsequent expression of clinically manifest autoimmune disorders [64, 112]. (b) Bystander activation effects may occur during stimulation of the innate immune system by PAMPs. These include LPS, dsRNA, and bacterial lipoproteins which result in activation of the innate immunologic mechanisms with increased expression of a number of co-stimulatory molecules, cytokines and chemokines [118]. IFN-γ and IL-15 produced by such cells can result in activation or proliferation of CD8+ CD44hi+ effector or memory T cells. In addition, after antigen exposure, Th1 cells produce IFN-γ in response to IL-12 and IL-18. Human memory cells of the CD4+ phenotype have also been found to be activated by similar cytokine pools. All these events have been observed to occur independent of TCR signaling [119]. Such antigen-independent T cell activation has been proposed as a major mechanism in the evolution of tissue damage in rheumatoid arthritis [120], and possibly in other autoimmune disorders. It is possible that previously dormant, inactivated or tolerized autoreactive T or B lymphocytes are
activated by TCR-independent and immunoglobulin receptor-negative effects, which are mediated by several cytokines including IL-2, IL-12, IL-15, IL-18, IFN-γ, and IFN-β. (c) In addition to the antigen-independent activation of the T and B cell repertoire, there is evidence to suggest that pathogen-specific Th1, Th2, CD4+ T cells, B cells and CTL can also cross-react with self peptides and other autoantigens, to result in the development or aggravation of underlying autoimmune disease processes [68].

**Concluding Remarks**

It is evident from the information reviewed here that mammalian immunologic homeostasis is a function of a complex interplay of the external environment (consisting of a multitude of antigens, microbial organisms, other naturally acquired or induced macromolecules) with a mammalian host richly endowed with components of nonspecific barrier mechanisms, innate immune functions and components of adaptive immunity at mucosal surfaces and in different systemic tissues. The nature and the state of immunocompetence in a mature host is a reflection of continuing evolutionary developments driven to a large extent by the changing external environment. Of particular importance is the mucosal microflora and its influence on the regulation of systemic and mucosal immunologic reactivity. In contrast to the adult, the neonate exhibits certain unique immunologic characteristics including: low levels of innate immunity; reduced expression of proinflammatory cytokines; impaired neutrophil, macrophage, DC and other antigen-presenting cell function; altered cellular and T cell-dependent antibody responses, and a relative increase in eosinophils and enhanced mechanisms of apoptosis. This is particularly important because the normal neonate is delivered rather suddenly from an essentially microbiologically sterile uterine environment into the perinatal and postnatal environment loaded with a diverse spectrum of pathogenic and non-pathogenic microorganisms. Despite the immense exposure and the high potential for acquisition of and colonization by such organisms, the development of clinically apparent disease in a normal neonate is an exception rather than the rule. Nevertheless, some infections are unique to this age and may set the stage for subsequent chronic disease outcomes. One such example is the development of otitis media in early childhood. The development of infections with viruses, such as RSV, influenza and rhinoviruses appear to be important determinants of otitis in the first year of life. Such infections appear to set the stage for subsequent acute or chronic suppurative infections with *Streptococcus pneumoniae* and other bacterial pathogens. In a series of elegant investigations, it has been observed that the development of suppurative otitis media in young children up to 1 year of age was directly related to nasopharyngeal colonization with the same organisms well before the development of otitis. Increased rates of colonization in
nasopharynx with *S. pneumoniae*, *Moraxella catarrhalis* and non-typable *Haemophilus influenzae* were characteristically seen in subjects who subsequently developed suppurative otitis media with the same organism [121–123]. Other studies, reviewed in more extensive detail elsewhere at this symposium, have provided very impressive evidence to suggest increased mortality from infections later in life secondary to nutritional impairment or other environmental insults in early childhood. Such increases in mortality and morbidity may have been related to malnutrition-induced smaller thymic size, reduced TCR excision circles, a marker for thymic output of T cells, reduced thymic output, and possibly reduced IL-7 activity [124, 125]. Other investigators have suggested that impairment of antibody response to polysaccharide antigens in adult life may be related to prenatal and early childhood nutritional impairment [126]. Acquisition of normal or abnormal mucosal microflora in the early perinatal and neonatal period may be one of the most critical environmental factors underlying the later development of such immunologic abnormalities in the host.

It appears that available environmental microflora and its colonization of the mucosal surfaces is significantly influenced by the immunocompetence of the host and the level of community sanitation and personal hygiene. Such societal approaches can significantly alter the level of bacterial, viral and helminthic load required to generate balanced physiologic immune responses or expression of clinical disease. Reduced exposure to Th2-promoting helminthic infestations in the gut may account for Th1 hyperreactivity and related inflammatory bowel disease. On the other hand, reduced exposure to Th1-promoting commensal pathogens in the neonatal period and infancy, use of antibiotics, and lack of breastfeeding may result in increased Th2 responses in the respiratory tract with increased allergic diseases. Mucosal DCs and regulatory T cells may be critical in directing and controlling T cell responses in such altered states.

Recent animal experiments have shown that sustained exposure to helminths results in protection against experimentally induced colitis and other immunologically mediated diseases. Induced infestation with parasitic agents has also been employed to treat (with some success) ongoing inflammatory bowel diseases. Such infestation induces mucosal Th2 responses, with expression of IL-4, TGF-β and IL-10. Helminthic infestation also appears to induce regulatory T cells which limit effector T cell function. These effects seem to be related to the induction and functional activation of different TLRs (in particular TLR-4) in the mucosal tissues [127]. Why different TLRs are activated with different stimuli remains to be determined. However, a large number of environmental factors including the nature of microbial colonization in early life, genetic polymorphism of the TLR, and engagement of signaling intermediates during TLR activation may be related to such differential activation processes. Activation of interferon-regulating factor-3 has been shown to result in increased expression of IFN, while activation of the
NF-κB pathway results in increased expression of inflammatory cytokines and chemokines.

Based on the evidence available to date, it has been proposed that signaling through TLR preferentially favors development of Th1 (IFN-γ, α) responses necessary for protection against microbial pathogens. On the other hand, development of Th2 (IL-4, IL-5, IL-13) responses favor protection against parasitic agents. The decision to express Th1 or Th2 cytokines appears to be made by the host very early during the course of the immune response to an infectious agent. For example, in studies with *Mycobacterium leprae*, it has been observed that induction of the Th1 response is associated with the development of tuberculoid leprosy which is characterized by a low mycobacterial organism load and very few systemic manifestations. On the other hand, Th2 responses are typically observed in lepromatous leprosy, which is associated with a very high mycobacterial load, and severe systemic disease [128].

The complex nature of TLR activation, its association with the type of mucosal microflora and the nature of the subsequent Th response (Th1 vs. Th2) may be the principle mechanism underlying the development of protection against or the pathogenesis of a disease process. Other important considerations which may influence the effects of infection include the temporal relationship of the infection to the development of immunologic disease; age at the time of infection; dose and severity (antigenic load) of the infection, and the possible route and sites of localization of infection [128].

Based on the information summarized in this report and in other reviews presented here, it is very likely that the interplay of commensal microflora, pathogenic microorganisms, helminths, prebiotic factors and other dietary immunomodulators during the unique immunologic window in neonates and early infancy, with the host’s innate immune system, is critical in determining the subsequent nature of the adaptive immune responses, the development of protective immunity, or the induction of immunologically mediated (allergic vs. autoimmune) disease process.

These observations provide an immunologic basis to the ‘hygiene hypothesis’. This concept, initially proposed by Strachan [129] in 1989, is based on careful observations of household size and local hygiene as risk factors for hay fever, and utilizes even earlier observations by Gerrard et al. [130] for serum IgE levels in two hygienic settings.

The implications of these and more recent observations are discussed in detail elsewhere at this symposium. However, it is interesting to note that during the past century, mankind has witnessed a dramatic reduction in the global burden of many infectious diseases and discernable improvements in the overall quality of life. These changes are in a large part due to the introduction of community sewage systems and sanitation, better nutrition and most significantly due to the introduction of childhood vaccines for available vaccine-preventable infectious diseases. Paradoxically, these successes have
also been accompanied by the increasing use of antibiotics and the evolution of large numbers of antibiotic-resistant organisms with severe or fatal disease outcomes in nosocomial and community-based settings, and the identification of new disease-producing agents or the emergence of infections with agents previously considered to be nonpathogenic, and the development of new immunologically mediated disease processes. These events have in turn fostered a worldwide preoccupation with extreme personal hygiene with the introduction of newer antiseptics, antimicrobials and other modalities designed to kill bacteria and other environmental microorganisms.

Regardless of the advantages and disadvantages the mammalian host has acquired over the past 3 billion years of evolutionary changes, it is difficult for man to turn the clock back and revert to the hygiene and sanitation prevalent in earlier decades when allergy and autoimmune diseases were not a major health hazard. However, it may be possible to develop newer modalities which may foster some respect for our environment and mucosal microflora and a reevaluation of the contemporary societal attitudes which have contributed to the development of abnormal or altered immunologic homeostasis.

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External Environment and Mucosal Immunity

Dr. Bier: You mentioned that allergic diseases are relatively recent in evolutionary history, and even though lots of new antigens have been introduced in the last several centuries, it seems to me that the great bulk of earth-like antigens have been present for vast periods of time; bacteria have been present for vast periods of evolutionary history. So my two related questions are firstly what is the evolutionary adaptive advantage of re-inventing the acquired immune system in every generation, and secondly, since does not persist, does this mean that those environmental agents like the bacteria, etc., have actually developed systems that we don’t understand that prevent them from persisting?

Dr. Ogra: I don’t know the real answer to the first question. It would seem that the innate immune system is the first line of defense with a rather limited repertoire for pathogen interactions, somewhere in the range of 100–1,000 receptor sites. The adaptive immune system must have evolved to provide more breadth to the process of immunologic interactions to the host in order to deal more effectively with the ever-changing exposure to a large environmental antigenic reservoir. This is supported by the fact that the receptor repertoire in the B and T lymphocytes is somewhere in the range of hundreds of billions of sites.

With regard to the second question, there is very clear specificity which has evolved through the evolutionary process for interactions between the host and the organisms. Under physiological conditions, the patterns of mucosal colonization in selected mucosal sites are highly restricted to certain species of organisms and seem to be directed both by the host as well as by microbial factors. For example, there are
proteases which are generated by certain organisms, and there are specific ligands generated by the host and designed for binding to such specific bacterial ligands. This relationship has evolved over millions of years.

It should be pointed out that no microbial agent is totally benign. For example, many probiotics are considered benign, but under certain nonphysiological conditions these ‘benign’ agents can be highly pathogenic and can produce very severe disease. The pathogenic nature of the microbial agents and their relationship to the host is a dynamic one, and often a compromise which favors the survival of the host as well as the microbe.

Dr. Björkstén: I agree that parasites may regulate immunity as you suggested. I will show similar data on tolerance induction in infancy from Estonia where there are no parasites. My suggestion is that the supposed effects of parasites may in fact be due to the very broad and diverse microbial spectrum that you have in Africa. Fifteen years ago Eastern Europe was in some respects Africa minus parasites. B The ‘hygiene hypothesis’ is a most unfortunate term because it suggests that poor hygiene would be good for your health, which nobody in his right mind would argue for. It has been suggested that vaccinations could be risk factors for the development of allergy. The fact is that vaccines are very good for what they were intended but they are neither good nor bad in relation to allergy. We studied Bordetella pertussis which is a well-known Th2 stimulant. There was no relationship with allergy development, except in the placebo group where unvaccinated children who caught whooping cough had an increased incidence of allergy.

Dr. Ogra: Let me respond to the issue of vaccines as a risk factor for disease. There are currently about 800 websites directed against the use of vaccines. There are many individuals who feel very strongly that we should not vaccinate any child at any time. One of the arguments put forth is that some naturally acquired infectious diseases are associated with or can cause the development of certain autoimmune processes or allergies. Such a rationale implies that using vaccines in very early childhood results in unnatural exposure to many infectious agents early in life. Such an exposure can subsequently produce autoimmune disorders or allergies later in life. However, the large number of epidemiologic studies and other experimental data available at this time strongly indicate that currently licensed vaccine antigens are not the cause of any autoimmune or allergy disease identified to date. Most vaccines are absolutely safe and not associated with any long-term deleterious effects.

Nevertheless, there are data to suggest the induction of subtle or overt immunologic alterations after immunization with some vaccines. Studies with repeated immunization with DPT have demonstrated increased IgE levels in the serum, but such increased IgE activity has not been clearly related to the development of allergic disease processes in such vaccines. Furthermore, other non-vaccine components or possible contaminants present in earlier vaccines have been associated with the development of some even serious side effects. These include alum, mercury, antibiotics, egg proteins and possibly other adventitious infectious agents and tissue culture components. However, in the currently available vaccine preparations, these potential sources of hazard have been partially or totally eliminated. It is abundantly clear that the introduction of vaccines has been the single most important success story of modern human technology. In the past century this approach has eliminated diseases such as smallpox and significantly reduced the disease burden of infections such as poliomyelitis, measles, mumps, diphtheria, tetanus, Haemophilus influenzae type B, and many other serious and sometimes fatal infections. The available data suggest that the increasing incidence of allergic or autoimmune disease is not a reflection of vaccine-induced control of these infections nor the direct result of vaccine products employed.
**Dr. Björkstén:** No, I think DPT is a good story. We developed a test and were able to show that IgE antibodies are in this product, but it is an immunological phenomenon that has nothing to do with the disease. There is only one pertussis vaccine, which is not on the market, that has a purified component and it really induced strong IgE antibodies.

**Dr. Walker:** This is a complex topic that has evolved very rapidly over the last few years. A lot of energy has been directed at the prevention of immune-mediated disease, and most of that has been directed towards the neonatal period. What do we know about dealing with disease once it has already expressed itself in an immunologic fashion? Are there ways that we can potentially reverse the process and turn the disease off?

**Dr. Ogra:** Excellent question. One approach recently employed has been to induce specific oral tolerance, even after expression of the clinical disease, employing the use of sublingual mucosal tissue sites for antigen production. Dr. Björkstén may have additional recent data to expand on this approach.

**Dr. Björkstén:** At least from the allergy field, various anti-IL-5 and IL-10 receptors have been tried and were largely failures because of the side effects. At that stage they had such broad ranges that it was not possible to interfere because then you are really interfering with the entire cascade. The idea is that interference must be early, and early at this stage, at least from the allergy immune responses, is probably within the first 3 years. We had a paper in *Nature Immunology* [1] on that; all these compounds may theoretically be very good very early, but they certainly are not useful at this stage in established disease.

**Dr. Ogra:** Some of you may recollect an elegant study done by Sulzberger and Chase several decades ago, in which they demonstrated the induction of tolerance to systemic immunization with a hapten by prior oral feeding of the hapten antigen. This is commonly referred to as the Sulzberger-Chase phenomenon, and is probably the first documentation of oral tolerance as we know it today. Whether such tolerance induction can prevent the development of disease or influence recovery from an established disease remains to be determined.

**Dr. Prentice:** I want to return to the question that Dr. Bier asked: what is the adaptive reason for not maintaining all those specific memory responses in the host? Dr. Hanson has always told me that it would simply be impossible to do, that it would need an absolutely enormous genome and the cost of maintaining it would be phenomenal, and even if we had a single T cell which could recognize all the possible antigens, then that would require greater than the entire body mass of our whole body. So I think evolution is a compromise. We can only maintain a few of the innate protective mechanisms and it is impossible to maintain all the memory ones.

**Dr. Ogra:** It seems that memory is not life long. Re-exposure to the antigen via the environment is important to keep on reinforcing the processes of down- and/or upregulation of specific immunologic activity. It is not known whether memory is a function of persistence of the antigen-reactive cells, recruitment of a new cell population after antigen re-exposure, or to persistence of the antigen itself.

**Dr. Hanson:** As to the remarkable evolution of the immune system as you get old: it may be that we keep what is useful, simply by being continuously exposed and boosted with the antigens around. We do away with the rest, not affording it, not needing it, doing quite well most of the time. Is that what happens?

**Dr. Ogra:** That’s a wonderful question, and I don’t know the answer. I think there is something which nature has learned over millions of years of evolutionary biology, and that is senescence. Every life form has to die eventually. We all die as a collective organism because the individual cells die. What may regulate the programmed senescence or apoptosis of the lymphoid tissue as we get old may be a function of the environment to a large extent. Dr. Bier, do you have any additional thoughts on this issue?
Dr. Bier: I have no idea because it is not my field. I would like to comment on two things, one is Dr. Prentice’s remark which I think is a highly plausible approach. I would argue that there are a vast number of antigens, common things, that one could probably develop a relatively generic repertoire to deal with. Perhaps we don’t need to deal with the genome, we may need to deal with some other ways of preserving genomic memory, such as epigenetic things that are turned on and off, methylation, I don’t know. It just seems to me that there are possibilities there. The other is the issue of the hygiene hypothesis, the cleaner environment. We are just in a somewhat less dirty environment, we are not in a clean environment, and that is the problem I have with that particular approach.

Dr. Wilson: As you showed on one of your early slides, evolution really doesn’t seem to give a darn about anyone over about 25 – it never has. Evolution is driven by reproductive fitness and is thus focused on having people survive and be fit through their years of greatest reproductive capacity. There is one possible caveat to this notion – if post-reproductive age caregivers help to keep their grandchildren alive, there might be some minor evolutionary pressure for fitness at an older age, particularly for women. The second point relates to Dr. Bier’s comment. If you look at evolution and ask how this informs your question, it is only in bony fish and higher vertebrates where one sees an adaptive (antigen-specific) immune system in its traditional sense. Pancer et al. [2] have shown that jawless fish have generated an adaptive immune system through an alternative strategy (the use of variable receptors formed from leucine-rich repeat domains rather than immunoglobulin domains as in higher vertebrates), a form of convergent evolution. Lower phyla, some members of which are long-lived, have only innate immunity, but this form of host defense seems to be sufficient for them to thrive. What then accounts for the necessity of adding a highly complex adaptive immune system, which carries with it the risk of autoimmunity and cancer, and which also requires that you need to develop and ramp-up a specific adaptive immune response each time you encounter a new type of pathogen? This issue is a matter of ongoing speculation.

Dr. Barker: I would like to comment on the hygiene hypothesis and on Dr. Bier’s remark that the changes in hygiene have been really quite slight. I am guilty of inventing the term ‘hygiene hypothesis’ as an explanation of the epidemic of appendicitis which followed the introduction of running hot water into housing in Western countries. The reason for producing that hypothesis was as a dietary explanation for the huge rise in appendicitis, from being a nonexistent disease around 1900 to being the commonest cause of child death in rich people around 1920. There was a time when 1 in 5 people in Scotland had acute appendicitis during their lives. The reason of bringing it forward was because the dietary explanations for appendicitis had holes all over them. The changes in hygiene which followed the availability of running hot water in homes must have been quite considerable, and if they weren’t then we must be very sensitive to small changes because it generated a massive epidemic which killed huge numbers of children.

Dr. Giovannini: What is your opinion on functional compounds having a major effect on human milk, especially with regard to formula with prebiotics and probiotics?

Dr. Ogra: That is an area of intense investigation at this time. I already mentioned that both Peyer’s patches and cryptopatches can be activated by probiotics. Both probiotics and prebiotics are present in milk. Do they play any role in the control of allergies? Possibly, yes. We may hear more about it from Dr. Björkstén. There is already significant evidence to suggest that probiotics influence the outcome of allergic rhinitis and some gastrointestinal diseases, including infections with rotavirus. However, as I pointed out earlier, there is no specific organism which is always beneficial and safe for the host, and there is no specific agent which is always fatal to that species.
normal evolutionary adaptation, all hosts and their adapted microbial flora must live together in some symbiotic compromise. However, if the ecosystem changes rapidly and the host–pathogen balance is lost, organisms which are normally non-pathogens will re-acquire virulence and the potential to produce disease. The same concepts may hold true for today’s ‘probiotics’. It would seem prudent to exercise caution and not to flood the mucosal ecosystem and replace the flora with any probiotics exclusively, at the cost of other existing normal mucosal flora.

References
