Natural Immunity
Edited by L. Bertók and D.A. Chow

Host Defence: An Interaction of Neuroendocrine-, Metabolic- and Immune Mechanisms in the Interest of Survival

ISTVAN BERCZI[1], LÓRAND BERTÓK[2] and DONNA A. CHOW[1]

[1]Department of Immunology, Faculty of Medicine, the University of Manitoba, Winnipeg, MB R3E 0W3, Canada; [2]"Fodor József" National Center of Public Health and "Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, H-1221 Anna u. 5, Budapest, Hungary

ABSTRACT

The term natural resistance refers to the capacity of living organisms to withstand injury caused by physical, chemical and biological agents that may be present in the external or internal environment. This protection is mediated by the natural, or innate, immune system, a multi-factorial and polyspecific defence system. Evolutionarily preserved germ-line receptors mediate the activation of natural immune cells that recognize genetically preserved, cross-reactive homologous epitopes (homotopes) in micro-organisms, cancer cells, virus-infected cells and distressed cells. In general, protection is based on balancing the defence mechanisms of the organism with the damaging effects of harmful agents. This defence comprises epithelial, secretory and endogenous mechanisms in addition to the cellular and humoural components of the natural immune system. In recent years, a continuing surge of exploration and discussion has helped to crystallize our appreciation of the molecular mechanisms of this innate system, their basis in evolution, physiological, pathological and behavioural significance and their regulation, in particular their intimate connection with the neuroendocrine system. In higher animals natural immune mechanisms are boosted profoundly during acute febrile illness leading to the release of pro-inflammatory cytokines, IL-1, TNF-alpha and IL-6, which in turn activate the neuroimmune regulatory network. The HPA axis and the sympathetic nervous system is activated and catabolism prevails. IL-6, glucocorticoids and cathecolamines induce the production of acute phase proteins permitting a rapid activation of phagocytic and cytotoxic mechanisms under the command of natural antibodies and other recognition molecules (e.g. C-reactive protein, endotoxin binding and mannose binding proteins). The acute phase response is a highly co-ordinated emergency defence reaction, which relies on the interaction of neuroendocrine, immune and metabolic mechanisms in the interest of maximum host defence during emergency situations, such as sepsis. In most cases febrile illness leads to healing and recovery, which attests to the effectiveness of the natural immune system and excites the desire for the benefits which should accrue from mastering the manipulation of this system.

1. INTRODUCTION

Innate or natural immunity has had a long and exciting past of which we are increasingly being made aware. The origins of the natural immune system are clearly buried in the evolutionary struggle for life. All living organisms, whether eucariotic, procariotic, unicellular or multicellular, from the lowest evolutionary stage right to the top, must have adequate defences against infections and other environmental threats. There is rapidly increasing knowledge of the various invertebrate defence systems, all of which depend on genetically stable, hard-wired innate mechanisms, or "natural" immune mechanisms, as they most frequently are called. It is also becoming clear that many, if not all of the natural defence molecules, which are present in vertebrates are also represented in lower animals. Most of the mechanisms involved are redundant and mutipotent, aimed at highly conserved, cross-reactive homologous epitopes, or *homotopes* for short. Homotopes are present on the targets of the natural defence system which may be microbes, infected – and cancer cells, as well as on other external and internal targets, as presented in this volume [1–3].

The evolutionary connection of our natural immune system to that of lower animals is truly fascinating. For instance, bacteria are known to express heat shock proteins, which must serve their survival under difficult environmental circumstances. In higher animals and man, heat shock proteins have similar functions and are intimately associated with steroid hormone receptors. Steroid hormones play important roles in the stress response [4,5]. Fungi make antibiotic substances, which miraculously work well in higher animals and man to fight bacterial infections. Antibiotics may be regarded to be analogous to defensins, and other antimicrobial molecules in our natural immune arsenal. Ciliates, which are the most primitive unicellular animals, are phagocytic organisms. Do they eat, digest and, therefore, destroy their microbial enemies? We know little about this, but our own phagocytic cells are certainly capable of eating away our enemies and much more.

Multicellular plants, as well as animals, must possess an effective system for organization and adequate defence mechanisms in order to survive and withstand environmental challenges and infectious agents. We share adhesion molecules with plants as attested by the stimulatory effect of plant lectins, such as phytohemagglutinine and concanavalin-A, on lymphocytes [6]. The existence of plant derived hormones, contraceptives and many remedies testifies further for our physiological and pathophysiological ties with the *plant kingdom.*

One striking conclusion, which is apparent from this book, is that the natural immune system serves well and protects most species of the *animal kingdom* with its enormous variety of defence mechanisms, whereas adaptive immunity exists only in a small minority of animals. If most species rely on innate mechanisms and survive well and prosper, this must speak forcefully for the enormous potency and versatility of this system. It is also clear that cytokines predate the adaptive immune system and so do neuropeptides predate the development of the central nervous system (CNS). Neuroimmune interactions also existed prior to the development of the central nervous system [7]. It is also regarding the innate system, as it is for the adaptive system, that immune mechanisms play an important role in normal physiology and pathology [8–10].

Today immunological host defence may be categorised into three major constituents: (a) species-specific resistance, (b) natural immunity and (c) the adaptive immune response. Species-specific resistance depends on factors defined by evolution. For instance, pathogens are capable of causing disease in some species whereas others remain refractory [11]. In contrast, natural immunity is subject to changes according to the environment of the individual and represent a long-recognised, important but largely unresolved challenge in medicine. Much more is known

about the adaptive immune response which exerts exquisite specificities towards antigenic determinants or epitopes of infectious agents and of other antigens. This has been the overwhelming subject of interest in modern immunology, with specificity the dominating concept in the study of immunity, so that we have learned much about the adaptive immune system [12]. Specific responses are detected more easily and the results may be presented in a quantitative form which offers to the scientist a more reasonable chance for success. It has been much more difficult to study natural immunity in spite of the fact that many of the cellular and immune factors that are involved in this system have long been recognised [13].

One may suggest without hesitation that the problem of natural immunity is one of the most important challenges in modern medicine. It is apparent that immune function, including natural immunity, is influenced by numerous medical interventions and other factors that include nutrition, surgical procedures, various forms of injury, ionizing radiation, chemotherapeutic and immunosuppressive drugs, environmental pollution and harmful lifestyle (incorrect nutrition, alcoholism, smoking, drug abuse, lack of exercise, etc.). These factors may decrease natural immunity, which could lead to disease or even death caused by facultative pathogenic organisms that are harmless to healthy individuals.

The AIDS epidemic has stimulated interest in natural immunity. It became apparent that AIDS patients died of secondary infections as a rule and these were due to the profound immunosuppression caused by HIV. Similar observations were made in cancer patients treated by chemotherapeutic agents and ionizing radiation, which suppress immune host defences. This fact emphasized the importance of natural immunity and the need for immunological rehabilitation and stimulation in such patients. It also became clear to immunologists that natural immunity was much more significant than previously recognised and that it deserved much more attention.

The phenomenon of natural resistance plays an important role in biology by allowing for the selection of individuals that are most resistant to disease. In our time, natural selection is curbed by human intervention. This demands further attention to the enhancement of immune mechanisms, which make it possible to save individuals who would have succumbed to disease in earlier times.

Clearly, we are just beginning to recognize the enormous complexity, efficiency and importance of this system in the Biology of animals and man and there is a lot to be clarified for a thorough understanding of natural immunity. The rationale for the evolution of this system is the focus of animated discussion and conjecture.

2. HISTORY: IMMUNE DEFENSE VERSUS SELF-ORGANIZATION

The concept of resistance to disease must have evolved simultaneously with the recognition of health and disease during prehistoric times. A decrease in resistance resulted in disease, which occurred in the weak and infirm as a rule. In spite of the common occurrence of disease throughout history, the underlying mechanisms remained unexplained for millennia. The scientists and physicians of ancient times were not able to define adequately the concept of resistance. Before the discovery of microbes, a number of factors (e.g. extreme cold or hot weather, starvation and environmental factors) were linked with the occurrence of certain infectious disease outbreaks or epidemics, often as a consequence of wars.

Experimentally *Pasteur* made the first observations with regard to the association of chicken cholera and abrupt changes in the weather. *Robert Koch* studied anthrax infection of chickens

after forcing them to swim in cold water. These experiments indicated that environmental factors have an influence on resistance to disease. Nevertheless the exact definition of resistance was not put forward till our time. The situation is somewhat similar to the one described by János in the Book of "Phenomena": 'two people sat on the roof, one was taken, the other was left behind'. In other words, it was generally recognised that during epidemics in a human or animal population some individuals always remained healthy. This occurred even when the majority of the population succumbed to disease. The ability of certain individuals to face deadly diseases was defined as *resistance*. No further distinctions were made in this respect. The term *immunity* was also used to describe the resistance of an organism against infectious disease [13].

2.1. Inflammation and phagocytosis

While Cornelius Celsus (30BC-AD50) has been attributed with the first description of inflammation [14], in thinking about the development of our current understanding of natural immunity, the formative work of Metchnikoff is often cited, with thanks to Alfred Tauber for keeping the spotlight on Metchnikoff [15–18]. As described by Tauber, Chernyak and Podolsky [rev in 16,19], Metchnikoff's observation of the process of inflammation around thorns in starfish larvae led him to propose the "phagocyte as the amoeboid mediator of cellular immunity" in an expansion of the phagocyte aboriginal function of "eating to feed" to "eating to defend". This concept provided a basis for the investigation of cellular immunity and a counterpoint of debate for the subsequently developed (immunochemically focused) school of humoural immunity [17]. Further, according to his interpetation of Darwinism, Metchnikoff postulated a fundamentally disharmonious concept of the living organism, in contrast with the view of individual health at the time, a condition of harmony [19]. Metchnikoff considered that "embryological development proceeds with cell lineages that are potentially in competition", disharmonious and required "harmonizers", phagocytes, to decide which cells would survive [quoted as in 19]. Thus, phagocytes actively defined what would later be referred to as immune "self". One could imagine that the generation of spontaneous mutations could act as a 'disharmonizing' process. Metchnikoff also argued that phagocytes would continue to actively define an organism in the adult state and that phagocytes would also defend the host (all in the interest of survival !). The latter led to his *Bacillus bulgarus* therapy, to replace the toxic flora of the large intestine with exogenous "friendly" microbes, a for-runner of current-day probiotics and driver of the yogurt industry [19].

2.2. Self/non-self: early days and ancient times

In the history of the immune 'self' controversy outlined by Tauber [18], the idea that the immune system distinguished between self and non-self was implicit in Metchnikoff's phagocytosis theory, and was formally expressed when Burnet introduced the ideas of self, and self-non-self discrimination leading to his proposal of the clonal selection theory in 1959 [20]. This shifted the arbitration of self/non-self from phagocytes to the clonally selected T and B lymphocytes of the adaptive immune response. Jerne's (idiotypic) network theory of the immune system published in the early 1970's, proposed a highly integrated and self-sensing lymphocyte system [21]. The system knows only itself and perturbation of the system is required for activation. While in the Burnet model, defense of self was the foundation of immune reactivity, Jerne's self-sensing network challenged the concept of immune 'self' and expanded the role of the immune system to physiological functions.

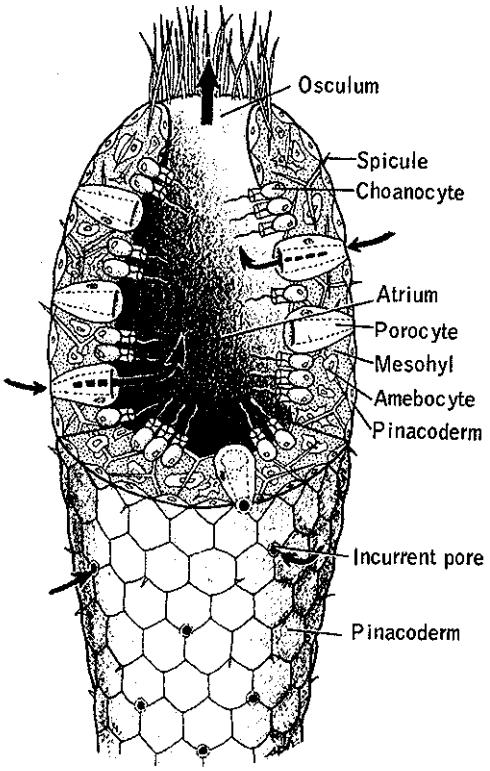

Figure 1. A partially cross-sectioned asconoid sponge [adapted from Ref. 22].

As is obvious from the above overview, immunologists have thought for a long time that self-recognition was a feature exclusive to the immune system. However, zoologists have long established that self-recognition was easily demonstrable in the most primitive multi-cellular animals, sponges (Fig. 1). Sponges control their morphogenesis, cell proliferation and differentiation. They preserve their species characteristics and have immune defences as well. They are capable of rejecting grafts from other species of sponges. Phagocytic cells provide their immune defence. Sponges were disintegrated by passing them through a screen. When brought together under proper conditions, the cells re-aggregated with cells from the same species, but not with cells from other species, so as to form functional sponge units, which could vary in size but with the maintenance of function. Clearly, these seemingly loosely aggregated cells behave as a highly co-ordinated morphogenic regulatory system. Sponge cells will grow and differentiate into functional cells according to their topographical localization [22]. Similar observations were made in higher animals. Cells from different anlages of the amphibian embryo were mixed and they were able to sort out into a pattern that resembled the initial organisation of embryonic tissue. Such aggregation experiments were also performed with embryonic cells from birds or mammals. Cell adhesion molecules present in embryonic tissue mediate such re-aggregation and play a key role in morphogenesis [23,24].

Embryonic morphogenesis is regulated by cell-to-cell contact and by diffusible mediators. Adhesion molecules are non-diffusible, hence they are capable of signalling single cells very specifically. It is very clear from embryonic development and from antigen-induced lymphocyte proliferation that adhesion signals are dominant over growth factor signalling. This is an obligate

requirement for morphogenesis, which is based on the positional relationship of cells/tissues to each other. Adherence signals determine, according to the local tissue/organ requirements, whether or not the cell is going to divide, differentiate and take up a function, or simply be on standby (survive), or perhaps be committed to the pathway of programmed cell death (apoptosis). Concentration gradients of tissue hormones and cytokines are important for morphogenesis during embryonic development [6]. This means that only certain cells will divide at any given time, while others will go into differentiation and take up the appropriate function according to their location in the body, or be on standby (stem cells, as well as differentiated cells) or may even be eliminated. Therefore, the systemic growth stimulus is modified according to the local needs, so that the morphological and functional integrity of the organism is maintained at all times. Growth hormone (GH) is well recognised as a hormone capable of stimulating the proportional growth of all tissues and organs. This dominance of local regulatory mechanisms over the systemic GH signal assures the development of a fully functional animal or human being.

Injured nerve cells in the CNS can be re-induced to grow axons and establish functional connections if exposed to non-neural elements of the peripheral nervous system [25]. This finding indicates that in adult tissues that lost their capacity to grow, stromal adherence signals are capable of inducing growth and regeneration.

Plants show a remarkable morphological and functional differentiation. Some proteins extracted from plants and collectively named lectins [26,27] activate animal cells, especially lymphoid cells for proliferation and function, including immunoglobulin secretion, cytotoxicity, helper or suppressor activity. Therefore, plant lectins function as regulatory molecules on animal cells and probably fulfil similar functions in the plants as well. Animal tissues also contain lectin-like adhesion molecules [27].

The restrictive power of cell-to-cell signalling is also fundamental to the adaptive immune response. Clearly, an antigen specific T lymphocyte clone must not proliferate unless it is triggered by the specific antigenic epitope in the context of self-MHC molecules. Without this restriction antigen-specific adaptive immune reactions would not be possible. Furthermore, MHC recognition by suppressor T lymphocytes and inhibitory receptors in natural killer and other cells serve as safeguards against the killing of normal non-infected and non-cancerous cells [28].

These facts indicate that adhesion molecules and locally active soluble mediators (cytokines) are required for organogenesis. It is likely that the same mediators are needed for the re-assembly of sponge cells after disintegration. Sponges exhibit a definite axis as vertebrates do, and it is also possible to define an imaginary head-tail orientation. There is no nervous system or endocrine system present in these primitive animals. On this basis, the principal regulatory circuits that are anticipated to operate in sponges and in similar primitive animals are shown in Fig. 2. During evolution the neuroendocrine system has been superimposed on this basic regulatory circuit, which reached its highest organization in man. So the situation has changed to the extent that the neuroendocrine system regulates the potential for growth and function (competence) in higher animals, whereas adhesion molecules and cytokines have the power to regulate competence locally according to the requirements in the various tissues and organs. Immune reactions, including natural immunity, are regulated according to this principle (Fig. 3). It is remarkable that in emergency situations (e.g. sepsis) the neuroimmune regulatory netwok is capable of selectively enhancing natural immune mechanisms, which provide instantaneous protection to the host, and at the same time to suppress the adaptive arm of the immune response, which is not capable of effective host defence under these conditions. This phenomenon has been coined as *immunoconversion* [1–3].

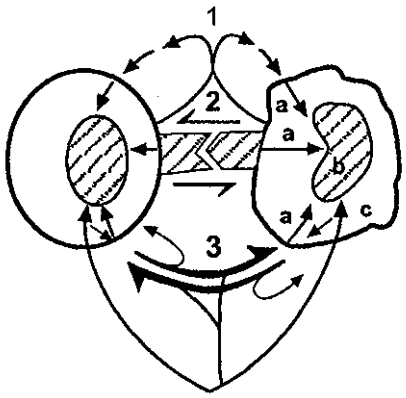

Figure 2. Hypothetical regulatory circuits of primitive animals without the presence of the neuroendocrine system.
1. Autocrine/paracrine secretion of competence (growth) factor. Competence is proposed to be a prerequisite of regulation by adhesion molecules and cytokines.
2. Adhesion molecules regulate the growth stimulatory signal according to the local requirements.
3. Cytokines complete the cell cycle. Some cytokines are of distant origin, which create gradients that are essential for axial and "head-tail" differentiation. Sponges already show such morphological differentiation.

Clearly, self recognition and self-non-self discrimination has been a prerequisite of the evolution for multi-cellular organisms and thus its development predates the development of the immune system in higher animals. The acceptance of self and rejection of non- self has been the rule right from the beginning for the phylogenesis of the multi-cellular animals and plants.

2.3 More mediators: the nude mutation

Beyond the abundant evidence for phagocytic cells, our understanding of the large number and variety of mediators contributing to the natural immune response was dramatically advanced by the discovery of nude mice. Mice bearing the nude mutation have a poorly developed thymus and lack of an effective T cell-mediated immune system. Yet, such mice did not develop an increased incidence of spontaneous tumours. This provided a strong impetus for the investigation of immune responses other than the thymus-dependent adaptive immunity [rev in 29]. This lead to a surge in research by many laboratories on the newly identified natural killer (NK) cells, which did not require previous exposure of the host to antigen, and exhibited instantaneous cytotoxicity towards tumour cells [30–32]. To a lesser extent observations of 'natural antibody' activity against tumours and apparently multi-specific, autoreactive antibodies from normal individuals also received (some) attention. The nature of immune 'self' became more complicated with the identification of such polyspecific and autoreactive NAb and with the demonstration of NK sensing MHC class I as "self" that delivers inhibitory signals and that 'missing self' leads to activation [33].

More recently, advances in cytokine research have allowed the study of dendritric cells, the major antigen presenting cell (APC) for naive T cell activation. This supported the search for the mechanism of the adjuvant effect, Janeway's 'immunologist's dirty little secret', needed to produce a vigorous adaptive immune response against a peptide antigen presented by MHC to TCRs. Investigations initiated by Janeway and Medzhitov [34], led to the pivotal finding that an array of Toll-like receptors on APC's could recognize different components on bacteria activat-

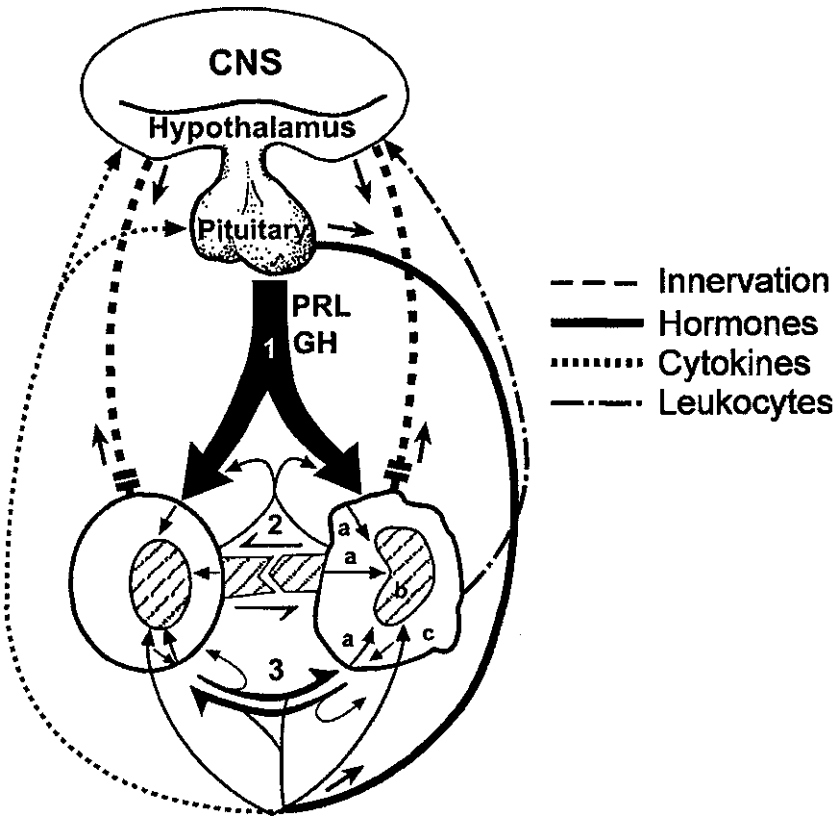

Figure 3. The neuroimmune regulatory circuit.
During evolution the neuroendocrine system has been super-imposed onto the basic regulatory circuit outlined above. The CNS, via the pituitary gland now controls systemically the competence signal for all organs and tissues that amounts to effective growth control of the organism. Adhesion molecules and cytokines remain in control of the local (e.g. positional) regulation of the systemic competence signal. The target organs/tissues provide feedback signals to the neuroendocrine system via innervation and by soluble mediators, such as hormones and cytokines. The neuroimmune regulatory circuitry is fundamental for the development and function of higher animals for their entire life cycle. It exerts physiological regulation and also fundamental to host defence, including regeneration and healing.

ing DC's to provide the needed costimulatory second signals for T lymphocyte activation. These discoveries brought the innate immune system attention, but it was chiefly framed as a supporter for the adaptive immune response.

2.4. An integrated system with expanded functions

Increasingly, investigators have viewed the immune system as a highly complex and integrated system, which communicates with the body in which it resides and self, non-self discrimination is less the issue, rather action is determined by the context of the interaction. Tauber [18], considers that the question of 'context' has been interpreted narrowly by some as being established at birth or shortly after, while for others (including Cohen, Coutinho, Grossman and Matzinger) the context is ever changing. The search to understand activation has focussed increasingly on, 1) the

nature of signals, which are ancillary to antigen recognition in the adaptive immune response, 2) the functional structure of the immune system in which they operate and 3) the relationships between the clonally selected T and B lymphocytes of the adaptive response and the mediators of the evolutionarily ancient innate immune response. Recognition of phylogenetic and functional relationships between the innate and adaptive immune systems has led to the concept of a more integrated immune system with a wider scope of function. Matzinger in particular, in defining the context of activation as "danger" rather than the discrimination between self and non-self, assigned the decision-making role for activation to the phagocytic APC. This proposal was supported by her observation that necrotic but not healthy or apoptotic cells released factors which could activate dendritic cells in vitro [35].

2.5. LPS at the core of innate immune research

The recently reviewed [36] long history of endotoxin study, provides a continuous thread (a fuse really) that starts before Metchnikoff with Hippocrates and informs our current concept of innate immunity. Beutler's and Rietschel's story of innate immune sensing focussing on microbial 'endotoxin' which in time became synonymous with lipopolysaccharide (LPS) a protein-free product from Gram-negative bacteria, cites three major milestones in the quest to understand how microbes create disease: the identification of endotoxin as a definable chemical species, the identification of soluble, host-derived mediators of toxicity (e.g., tumour necrosis factor, TNF) coupled with the idea that toxicity and protection were not readily separable, and recently the identification of the LPS receptor itself, providing evidence that many microbial toxins share mechanisms of action similar to endotoxin.

Additional bacterial components including some from Gram-positive bacteria exhibit endotoxin-like biological effects in mammals among them lipopeptides, lipoteichoic acid double-stranded RNA and unmethylated DNA with CpG motifs, and all are recognized by paralogous receptors [36,37].

3. EPITHELIAL, SECRETORY AND ENDOGENOUS HOST DEFENSE

3.1. Antimicrobial peptides

Epithelial tissues and granulocytes express numerous antimicrobial peptides called defensins and cathelicidins. These molecules were recognised initially for their broad-spectrum antimicrobial properties. However, now they are known to play a role in both the regulatory and effector arms of the innate immune system. These peptides modulate bacterial adherence ; complement activation ; fibrinolysis; steroid synthesis; mast cell activation; monocyte, neutrophil, mast cell, T cell and immature dendritic cell chemoattraction; cytokine expression; cytotoxicity; cell proliferation; angiogenesis; protease inhibitor synthesis; keratinocyte differentiation; proteoglycan synthesis; phagocytosis and Ca^{+2} mobilisation. Genes of the adaptive immune system encoding immunoglobulins and T cell receptors undergo a series of genetic amplifications and rearrangements. Homologous events involving genes of the innate immune system can be seen as generating a protective diversity promoting survival of a population or species. The mechanisms for this "genome instability" in innate immunity genes remain to be elucidated [38].

3.2. Endogenous cytoprotective mechanisms

Endogenous cytoprotective mechanisms protect the mammalian host against various forms of injury and noxious stimuli. Since these mechanisms are activated upon encountering potentially cytotoxic conditions, *Haem-oxygenase* and the heat shock response confer protection against a broad array of cytotoxic stimuli. The activation of the *anti-oxidant pathways* is critical to survival in an aerobic environment. *Hypoxia inducible factor* is a key transcription factor that directs the expression of genes necessary for adaptation to hypoxia and/or ischemia. *Nitric oxide* is a ubiquitous molecule that impacts a number of biological, physiological and pathophysiological processes [39].

3.3. Bile acids and natural resistance

Bacterial endotoxin is toxic, when given parenterally but is harmless upon oral administration. In naturally occurring *entero-endotoxaemic diseases* (e.g. shock due to sepsis or to other causes), endotoxin is known to absorb from the intestinal tract. If the common bile duct of rats was chronically cannulated (bile deprived animals) orally administered endotoxin was absorbed from the intestinal tract and provoked shock. This absorption was prevented by sodium deoxycholate or by natural bile. Bile acids split the endotoxin macromolecule into non-toxic fragments. This detoxifying detergent action of bile plays a significant role in host defence against infectious agents with a lipoprotein outer structure (e.g. "big" viruses). This represents a *physico-chemical defence system.* Bile deficiency and the consequent endotoxaemia are important components in the pathogenesis of certain diseases, such as sepsis, intestinal syndrome of radiation disease, hepato-renal syndrome, parvovirus infection, herpes, psoriasis, atherosclerosis, etc. Finally, bile acids may be used for the prevention and/or therapy of some clinical conditions such as the hepato-renal syndrome and psoriasis [40].

4. THE NATURAL IMMUNE SYSTEM

4.1. Natural killer cells: history and current status

Natural killer (NK) cells are a major component of the immune system, which play important roles in host defences against cancer and microbial infections. NK cells are distinct from T or B lymphocytes, with a characteristic morphology of large granular cells, and can be readily identified by characteristic cell surface molecules. They have the ability to recognise and rapidly kill a wide array of tumour cells and also virus-infected normal cells. NK cytotoxic activity can be strongly augmented by interferon, interleukin-2, and other cytokines. NK cells are major producers of some cytokines, especially interferon gamma [41]; they produce a neutrophil-activating factor and upregulate polymorphonuclear leukocytes to kill *Candida albicans* [42].

4.2. The reticuloendothelial system

During the nineteenth century varied cell types, which acted in host defence by phagocytosing foreign invaders were grouped collectively into the reticuloendothelial system (RES). The depression or blockage of the granulopoetic activity of this system has attracted considerable attention. Gadolinium chloride ($GdCl_3$), depresses RES activity and selectively suppresses or

eliminates the large Kupffer cells. Kupffer cell blockade modifies the immune response, exerts protective effects on anaphylactic and endotoxic/septic shock, and decreases the liver-damaging effects of several hepatotoxins and ischaemic reperfusion. Recent studies have elucidated the mechanisms by which $GdCl_3$-induced Kupffer cell blockade protects against a variety of hepatotoxic processes [43].

4.3. Effector mechanisms of natural immunity

Evolutionary approaches to the investigation of innate immune mechanisms has shown that monoclonal antibodies to human adhesion molecules react with earthworm, leech and sipunculan leukocytes. Many CD markers common to vertebrate leukocytes, especially to macrophages and natural killer cells are expressed. In earthworms, only those leukocytes which are positive are active as killers in cytotoxic responses, whereas larger, primarily phagocytic leukocytes are negative. The evolution of complement can be traced from sea urchins to the teleosts and tetrapods, exhibiting at each level a corresponding increase in the numbers of complement components and duplications in complement pathways. Invertebrates and vertebrates seem to possess common signalling molecules e.g. neuropeptides. These signalling molecules are immunomodulators in circulating blood. In vertebrates, release occurs during stress that triggers the hypothalamo-hypophyseal-adrenal (HPA) axis. Neuropeptides are conserved messengers that regulate innate immune responses in invertebrates and in humans. The evidence suggests that the cross talk between nervous and immune systems has an ancient evolutionary origin, which is essential to homeostasis [7].

4.4. Natural immune activation

Multiple recognition molecules are involved representing numerous structural families including, several lectin families, pentraxins, leucine-rich repeats, many members of the IgSF, integrins, scavenger receptors and the seven transmembrane receptor family. Invading pathogens exhibit a range of different repeating epitopes. Host molecules express a variety of receptors capable of recognising these epitopes and act in a combinatorial manner which confers specificity to the host response. The large number, diversity, and ancient evolutionary origin of these receptors argues for the essential nature of their functions. While providing a first line of defence against invading pathogens is clearly crucial for organism survival, evidence is accumulating that these same receptors also participate in essential physiological functions [44].

4.5. Signaling in natural killer cells

NK cells have the ability to recognize tumour- and virus-associated ligands. These cells express CD16, the low-affinity Fc receptor (FcR) for IgG. NK cells do not have a single type of receptor through which they recognize antigens. Rather, clonal subpopulations of NK cells differ in their expression of receptors that recognize a variety of ligands on target cells throughout the body. NK cell binding of these ligands initiates signalling cascades within the NK cell that control its response to the target [45].

4.6. Toll-like receptors

Macrophages are central in orchestrating the innate immune response to infection, which is not a trivial task: they must be able to discriminate microbes from self, and then initiate a proper response. The discovery of the Toll-like receptor (TLR) family of pattern-recognition receptors has provided insight into this kind of recognition. TLRs are expressed on macrophages and other innate immune cells, where they collaborate to read the molecular fingerprint of different microbes and initiate inflammatory signalling pathways. The TLR family is important in infectious diseases, and there is also evidence that they may play a role in autoimmunity and degenerative diseases in the central nervous system [46].

5. REGULATION OF NATURAL IMMUNITY

5.1. Leukocyte migration

Leukocyte migration is essential for reactions to inflammatory stimuli at various locations in the body. However, leukocyte movement is also crucial during non-inflammatory processes such as haematopoietic development and routine passage through secondary lymphoid organs, which is also required for effective antigen presentation. Immune defects occur in chemokine receptor deficient mice. Chemokines, their receptors and adhesion molecules play a key role in the regulation of the immune response during inflammatory and under homeostatic conditions. Leukocyte trafficking plays a role during developmental processes, for example in haematopoiesis and thymic maturation of T cells and in regulatory circuits that ensure immune surveillance and communication between the innate and adaptive components of immunity [47].

Leukocytes utilize an active process to halt chemotaxis and switch to effector activity, with the aid of the arrestin protein, which blocks chemotactic signalling from chemokine receptors and converts it to a signal for degranulation [48, 49]. The discovery of chemorepulsive activity mediated by CXCR4 provides a mechanism by which mature T cells may exit the thymus [50, 51].

5.2. Neuroendocrine regulation of natural immunity

Natural killer (NK) cells, γδ T lymphocytes and CD5+ B lymphocytes are key effector cells in the natural immune system. These cells utilize germ-line coded receptors that recognize highly conserved, homologous epitopes (homotopes). Under physiological conditions the natural immune system is regulated similarly to the adaptive immune system: growth and lactogenic hormones (GLH), insulin-like growth factor-I (IGF-I), insulin, leptin, some steroid (glucocorticoid at physiological concentrations, dehydroepiandrosterone and some of its derivatives) and thyroid hormones are stimulatory. The peptides of the hypothalamus-pituitary-adrenal axis (CRF, AVP, ACTH, αMSH, βEND) exert an immunosuppressive, anti-inflammatory and anti-pyretic effect. Opioid peptides and estradiol are immunomodulators that promote some immune activities while inhibiting others. High (pathophysiological) levels of glucocorticoids, progesterone and testosterone act as immunosuppressive hormones. Beta-adrenergic agents are immunosuppressive and anti-inflammatory, whereas cholinergic agents promote immunity and inflammation. Substance P and calcitonin-gene related peptide are pro-inflammatory and promote immunity, whereas somatostatin is an antagonist of these neuropeptides [52].

Mild infection or a sublethal dose of endotoxin elicits a brief elevation of GH and PRL in the

serum. Severe trauma, sepsis and shock results in the elevation of TNFα, IL-1 and IL-6 in the blood stream, the GLH-IGF-I axis is suppressed, whereas the hypothalamus-pituitary-adrenal axis is activated. LH, FSH, estrogens, androgens, progesterone, and thyroid hormones all decline during infection and endotoxin shock, as a rule. Leptin, insulin, glucagon, α-MSH, endorphin, and arginine vasopressin are increased during endotoxemia. A "sympathetic outflow" leads to elevated blood levels of catecholamines. Fever and catabolism prevails, whereas acute phase proteins in the liver, cell proliferation in the bone marrow, and protein synthesis by leukocytes are increased. This is an acute emergency reaction to save the organism after the adaptive immune system has failed to contain and eliminate the pathogenic agent. During sepsis and endotoxin shock, glucocorticoids potentiate the production of acute phase proteins and regulate pro-inflammatory cytokine production. Catecholamines also inhibit inflammatory responses and promote, even initiate, the acute phase response. Leptin regulates energy metabolism and it is a major stimulator of the immune system. If the acute phase reaction fails to protect the host, shock will develop and death will follow [52].

The acute phase response leads to *immunoconversion*, which involves the suppression of the T cell regulated adaptive immune system and the amplification of natural immunity. Natural antibodies, C-reactive -, endotoxin binding- and mannose binding proteins are boosted and serve as polyspecific recognition molecules for leukocytes. The natural immune system provides the first and the last line of host defence and its functional integrity and massive activation is largely dependent on the neuroendocrine system [52].

5.3 Natural immunity – Effect of exercise

Natural immunity is influenced by pharmacological agents, the environment, exercise and diet. Exercise induces increased circulating levels of a number of cytokines, especially IL-6, which is produced locally in contracting skeletal muscles and accounts for the arterial IL-6 concentration. In turn, IL-6 stimulates the production of a number of anti-inflammatory cytokines such as IL-1ra and IL-10 and also works in a hormone-like fashion. IL-6 also stimulates cortisol production and in the recovery phase of heavy exertion, a cortisol-induced shift in leukocyte subsets is seen. The dominant features in the post-exercise period are lymphopenia, neutrophilia and a markedly suppressed natural killer cell activity. In addition secretory IgA is inhibited [53].

Many clinical physical stressors (e.g., surgery, trauma, burn, sepsis) and environmental factors such as hyperthermia and hypoxia induce hormonal and immunological responses that have similarities to the cellular response to exercise [54]. Training at an intense level over many years can result in a chronic suppression of salivary immunoglobulin levels. The degree of immune suppression and the recovery rates after exercise are associated with the intensity of exercise and the duration or volume of the training [55]. The effect of acute hypoxia on lymphocytes resembles the effect of exercise [56].

The production of IL-6 from working muscles is further enhanced if muscle glycogen content is low. Carbohydrate intake during exercise attenuates the IL-6 production and consequently exercise-induced cortisol production and fluctuations in NK cells and neutrophils. A major new finding is that exercise-induced immune changes are not a secondary phenomenon to exercise-induced hormonal changes. Rather, muscle contractions induce the release of IL-6. By producing IL-6, muscle fibres are directly involved in exercise-induced immune changes, and exercise-induced cortisol changes can be viewed as a secondary phenomenon, which in turn leads to altered leukocyte subset composition. As IL-6 works as an energy sensor, it is also clear that dietary factors such as carbohydrate, may influence the immune response to exercise. It is

noteworthy, that a cytokine, previously known as a component of the natural immune defence, should now also be considered as an important player in metabolism [53].

5.4. Enhancement of natural immunity

Endotoxin injections produce endotoxin tolerance and elevate natural resistance. However, such injections may have serious side effects, such as high fever, hypotension and abortion. For this reason LPS injections are not suitable for the enhancement of natural immune mechanisms in endotoxin-sensitive mammalian species. Various techniques have been used (physical, chemical, etc.) for the detoxification of endotoxins while the beneficial effects were maintained. One of the best detoxification techniques is treatment with ionizing radiation. The irradiation of LPS with ^{60}Co (100–200 kGy) decreased its toxicity. Such radiodetoxified endotoxin (RD-LPS) preparations showed decreased toxicity, whereas the beneficial effects were preserved (150 kGy:TOLERIN®). Irradiation causes marked chemical alteration in LPS, such as the decrease of glucosamine, KDO and fatty acids. A single parenteral injection of TOLERIN® is capable of preventing various shock syndromes in experimental animals. Unlike endotoxin, TOLERIN has barely any hypotensive effect and pretreatment with this preparation can prevent practically all the haemodynamic changes induced by LPS. LPS plays an important role in the pathogenesis of the intestinal syndrome of radiation disease, which may be prevented by up to 70% in rats with RD-LPS pretreatment. TOLERIN retains the adjuvant activity of LPS and it is a good adjuvant for inactivated virus vaccines. TOLERIN can also evoke the regeneration of the immune system in irradiated animals. The decrease of natural immunity in immunodeficient or immunosuppressed patients is the most important cause of opportunistic infections that may lead to sepsis, endotoxaemia, pneumonia and so on. Boosting of natural resistance and the induction of endotoxin tolerance are important in such patients. RD-LPS could produce significant proliferation of lymphoid cells in germ-free animals, which are immunodeficient. Many other beneficial effects are exerted by RD-LPS preparations, such as the activation of macrophages and of the reticuloendothelial system, antitumour activity, etc. On the basis of these favourable experimental results, TOLERIN was tested on 350 surgical patients suffering from gastrointestinal tumours, on patients suffering from AIDS and on cancer patients treated with CYSPLATIN®. TOLERIN treatment prevented sepsis and activated bone marrow function in these patients [57].

6. PHYSIOLOGICAL, PATHOLOGICAL AND BEHAVIORAL SIGNIFICANCE

6.1. Physiological activities of the natural immune system

Recent research has revealed the extensive underlying physiological role of the innate immune system in the development and homeostasis of the organism. The impact on development is evident during embryogenesis and also during normal cyclical changes in reproductive tissues in the adult. Mediators of the innate immune system are essential for normal tissue renewal and healing, regeneration, air breathing, cell signalling and cancer control. Natural immunity contributes to the normal physiology of the organism in many and diverse ways arguing for an evolutionary selection centred on self-organization for survival [8].

6.2 Pathophysiological relevance

The natural immune response is a pre-programmed, poly-specific first line of defence that is primarily responsible for eliminating or containing pathogens at the site of entrance into the host. This evolutionary conserved system was described first in cells of the immune system. However, it became apparent this form of immune potential exists in various tissues, where its activation plays a significant role in host defence, autoimmunity, inflammatory disease and pathogenesis of sepsis-induced multiorgan dysfunction. It is possible that natural immunity plays a role in the aging process, and in tumour immunosurveillance [9].

6.3 Behavioural mechanisms in host defence

Behavioural strategies assist organisms to defend themselves against pathogens. Reflexive behaviours, like coughing and vomiting, can be instrumental in expelling pathogens from the body. The avoidance of excrement is an important strategy for minimizing contact with pathogens. Learned food aversions and the avoidance of stimuli previously associated with illness minimize contact with pathogens. Behavioural changes can be induced by immune activation, and it has been argued that this "sickness behaviour" may assist the organism to recover from infection. Immune activation is associated with the production of cytokines, some of which (most notably, interleukin-1, IL-1) have potent behavioural activities. IL-1 decreases several behavioural activities, such as food intake, and sexual activity in females, but not in males. Certain kinds of behavioural experiences, most notably stressful ones, may induce immune activation and cytokine production. Environmental stressors and immune activation produce some similar physiological responses: increased body temperature, and activation of the sympathetic nervous system, the adrenal medulla, and the hypothalamo-pituitary-adrenal axis, as well as brain catecholamines and indoleamines. These observations have led to the concept that pathogen invasion induces "immune stress". The physiological responses induced can assist the defence of the organism against infections, at least in part by changing behaviour. It is argued that learned and reflexive behavioural strategies, and physiological and behavioural responses to illness, are all important components of host defence against pathogens [58].

7 'MISSING SELF' AS A KEY TO INNATE IMMUNE ACTIVATION

The concept of 'missing self' first was coined with respect to the failure of activating NK cells due to the recognition of self-MHC [33]. This now appears as an emerging common principle in innate immunity. Sponges have phagocytic cells for defence. Another form of this strategy relies on cell expression of terminal sialic acid on cell surface molecules versus the lack of sialic acid on most microorganisms [59]. Sialic acid binding siglecs are inhibitory receptors bearing tyrosine-based inhibitory motifs (ITIMs). The lack of, or reductions in sialic acid on pathogens, some virally infected or transformed cells and apoptotic cells may act as missing self, allowing phagocytosis to proceed.

Complement, the major noncellular system of innate immunity in humans, exhibits wide ranging and potent biological activities which are under heavy regulation employing several different strategies. Since complement can react ubiquitously by binding covalently to self and non-self, regulator recognition of self can provide one strategy for preventing attack on the host. In this regard inhibition based on terminal sialic acid again contributes in the form of sialic acid-bind-

ing factor H of the alternate complement activation pathway, which promotes the inactivation of C3b and spares self cells [30,60]. Furthermore, additional complement inhibitors, which are broadly expressed and important in the control of complement activation on self cells are membrane expressed CD46 and CD55 (decay-accelerating factor, DAF) and the soluble and secreted C1 inhibitor and clusterin [61, 62]. All of these molecules are considered to constitute "don't eat me" signaling markers (SAMPs) [61]. These contrast with "eat me" markers in the form of soluble or secreted bridging molecules of the innate immune system binding to pathogen-associated molecular patterns (PAMPs) on pathogens and from apoptotic cell-associated molecular patters (ACAMP). Additional "don't eat me" signals result from normal host cell expression of CD200, CD47 and CD31, all of which engage inhibitory pattern recognition receptors (PRRs) and down-regulate phagocyte activities [61]. While complex, this form of control is well adapted for attack on pathogens not previously encountered. Furthermore, the lack of complement-receptor 1-related gene/protein y (Crry), a membrane-bound complement-regulatory protein structurally similar to decay accelerating factor (DAF/CD55) and membrane cofactor protein (MCP/CD46), resulted in complement deposition at the fetomaternal interface and fetal loss in mice [63], clearly supporting the essential nature of such 'self' expression in normal reproductive physiological processes. Thus, normal self must also include complement regulatory molecules.

8. APOPTOTIC CELLS AND THEIR PHAGOCYTOSIS

Our increasing understanding of cellular "corpse" generation and elimination [64] has contributed immensely to our appreciation of the scope of innate immune function. The concept of physiological cell death emerged from studies of animal development during the latter half of the 19th century [65]. However, the idea that a cell can activate a suicidal program of self-destruction (programmed cell death), which can be modified by external signals has only been developed during the last half of the 20th century, and this was considered essential for the development, homeostasis and integrity of multicellular organisms [65]. The description of cell death by apoptosis in the early 1970's provided a phenotype which became a focus for the investigation of homeostatic, 'physiological' cell death (cellular homeostasis) in normal and pathological tissues (e.g. cancer regression) [66]. Apoptosis was viewed as a prelude to the orderly removal of the non-viable cells by phagocytosis in the absence of inflammation. The molecular processes of apoptosis was investigated as an active mechanism of cell death induced by steroids, antibody-dependent cytolysis by lymphocyte killer (K) cells, NK cells and cytotoxic $CD8^+$ T cells consistent with its generalized utility in biology [67, 68].

The science of phagocytosis has undergone a revolution in the last few years from a descriptive to an analytical approach [71], and combined with advances in cytokine and chemokine research so has our appreciation of innate imunity. The demonstration that while phagocytosis of necrotic cells, clearly a danger signal, produces an inflammatory response, phagocytosis of apoptotic cells does not, provided further support for Matzinger's "danger'" hypothesis. Phagocytosis of apoptotic cells which have undergone a process of programmed cell death is at the core of many biological processes, pathological or physiological, which depend on the innate immune system.

9. NATURAL IMMUNITY AND THE NATURAL IMMUNE SYSTEM

While the innate immune system has traditionally been considered by many to have evolved to defend against microbial pathogens [59,70–72], others have proposed that the immune system did not evolve to fight infection [73,74]. Analysis of allorecognition challenged the paradigm that vertebrate immunity is pathogenetically focussed and directed support toward the idea that preserving individuality against the threat of invading conspecific cells (based on polymorphic compatibility molecules) was probably the driving force for all innate and adaptive immune systems and the defence function developed later [74]. Key molecules of the mammalian innate and adaptive immune systems were identified in sponges (Porifera) and some were considered likely to have acquired dual functions during evolution, acting first in adhesion and growth control and later in immune self/self- and self/non-self-recognition [75]. The bountiful evidence for the role of innate immunity in cell modulatory pathways of normal development reminds us of the limitations, folly even, of taking a narrow reductionist approach to understanding and investigating innate immunity. Investigation increasingly exposes the critical contributions of components of the innate immune system in normal physiological functions including, embryological development, reproduction, organ regeneration, and wound healing [8]. Antimicrobial peptides contribute as growth factors to wound healing and tissue repair. Phagocytes regulate angiogenesis by secreting growth factors [76] and by remodelling vasculature through macrophage-induced apoptosis of vascular endothelial cells [77,78]. Complement participates in crucial processes of normal development and organ regeneration [69]. NK cells and macrophages are considered important for a successful pregnancy contributing to implantation, vascularization, growth factor production for the placenta and trophoblast differentiation and parturition [79,80].

Several investigators have refuted the idea that the innate immune system is non-specific [14,74], rather it is extremely selective [61] and polyspecific, which is due to the multiple specificity of receptors (e.g. natural antibodies), and the crossreactivity of homotopes recognized by them.

Thus, a broad definition of the innate immune system which allows for extensive interpretation, would be an evolutionarily ancient, germline gene-dependent, self-organizing system (implying self-recognition) acting in the interest of survival. This would encompass all components of the immune system which fit this description (including T and B cells activated other than through specific clonaly-selected antigen recognition) participating in defence and self-organizing functions.

10. LINKS BETWEEN INNATE AND ADAPTIVE IMMUNITY

There are many examples illustrating that the natural immune system serves as a foundation, on which the adaptive immune system has evolved. Briefly, the macrophage, which is a principal co-ordinator of natural immunity, is also fundamental to adaptive immunity as an antigen presenting cell. Moreover, macrophages initiate the acute phase response, which leads to the inactivation of the thymus and the inhibition of adaptive immunity. B lymphocytes secreting natural antibodies may have an effect on any lymphocyte of the adaptive system that expresses Fc-receptors. Natural killer cells produce IFN-gamma and other cytokones that affect cells of the adaptive system. Defensins also affect adaptive immunity. Complement has been established as a vital link between natural and acquired immunity, profoundly augmenting the antibody response to T-dependent antigens [79]. In turn, activated T cells produce INF-gamma, the major

cytokine activator of macrophages.

11. CURRENT APPLICATIONS AND CHALLENGES

The scope of natural immunity is vast and complicated by extraordinary diversity, redundancy, cooperation and amplification. Research to date has established the legitimacy of the field but the surge in exploration must continue if we harbour any desire to live in harmony with our immune system. Nevertheless, our increasing understanding of the mechanisms of the natural immune system and its importance for the development of a strong adaptive response has provided a strong incentive to better understand the dynamic interplay between infectious agents and host defence in man [34] and to develop *new adjuvants* as a component of improved vaccines. Current approaches to immune potentiation and adjuvant design combined with vaccine delivery are rapidly moving the field forward [81]. Toll-like receptors, in particular, are being targeted in vaccine development and in cancer therapy [82].

The use of the innate immune system by itself has been proposed as a *biodefence strategy* for protection against a broad and largely unforseen range of microbial pathogens which may be employed in bioweaponry [83,84]. This could employ synthetic, conserved components of microbes recognized by the Toll-like receptors and other receptors of the innate immune system. Both prophylactic and post-exposure approaches appear to be working in animals to prevent or reduce infections.

The early suggestion of Metchnikoff to eat live bacteria to promote health has seen a dramatic rise in popularity and has developed into the field of *probiotics*, (eating live 'good' microbiota) and the related field, *prebiotics* (eating non-digestible oligosaccharides that target pathological microbiota) both with the idea of modifying the activity or composition of the endogenous microbiota [85–87]. While the gut has been the main focus of this probiotic research, endogenous microbiota found in other parts of the body, (e.g. urogenital tract, skin and nasopharynx) have also attracted attention and can be expected to yield success [87].

Clearly, our understanding of the *immunology of aging is of present and future economic and political importance* [88] considering the current large population of aging 'baby boomers' and future increases in the proportion of older people and in the age of old people that are generally predicted to happen. Since natural immunity seems to be less affected by age than the adaptive response (Salvioli et al, this volume), the ability to manipulate the natural immune system will be needed to maintain a good quality of life in old age. With regard to natural immunity, which is heavily regulated by the environment, both external and internal, probably the most important decision an individual can make is to choose a healthy life style in order to maintain the natural and adaptive immune systems for a long, high quality life.

REFERENCES

1. Berczi I. Neuroendocrine response to endotoxin. Ann NY Acad Sci 1998;851:411–415.
2. Berczi I, Bertok L, Chow DA. Natural immunity and neuroimmune host defence. Ann NY Acad Sci 2001;917:248–257.
3. Haeryfar SMM, Berczi I. The thymus and the acute phase response. Cell Mol Biol 2001;47:145–156.
4. Berczi I, Nagy E, Baral E, Szentivanyi, A. Sterid Hormones. In "Neuroimmmune Biology,

Volume 3: The Immune-Neuroendocrine Circuitry. History and Progress". Berczi I, Szentivanyi A, Editors, Elsevier, Amsterdam, 2003; pp. 221–270.
Gabry KE, Chrousos G, Gold PW. The hypothalamus – Pituitary – Adrenal (HPA) Axis: Mediator of the adaptive response to stress. In "Neuroimmmune Biology, Volume 3: The Immune-Neuroendocrine Circuitry. History and Progress". Berczi I, Szentivanyi A, Editors, Elsevier, Amsterdam, 2003; pp. 379–414.
Berczi I. The role of the growth and lactogenic hormone family in immune function. Neuroimmunomodulation 1994;1:201–216.
Cooper EL. Effector mechanisms of Natural Immunity: an invertebrate perspective. In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 103–121.
Chow D. Physiological Activities of the Natural Immune System. In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 311–329.
Salvioli S, Capri M, Fumelli M, Lescal F, Monti D, Franceschi C. Pathological relevance of the natural immune system. In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 331–350.
Berczi I, Szentivanyi A. Immune reactions. In: The immune-neuroendocrine circuitry. History and progress. Neuroimmune Biology, Volume 3. Berczi I, Szentivanyi A. Eds, Series Eds. Amsterdam: Elsevier, 2003; pp 315–377.
Bertók L. Experiments for the alteration of the species specific resistance by inhibition of protein-synthesis (Hungarian). Budapest: PhD Thesis;1964.
Gergely J. Phylogenesis and ontogenesis of immune response. In: Csaba Gy, editor. Actual problems of biology. (Hungarian). Budapest: Medicina, 1974;9–53.
Metchnikoff E. Immunity in infective diseases. Cambridge: Camridge Univ Press;1907
Revillard JP. Innate immunology. Eur J Dermatol 2002; 12: 224–7.
Tauber AI. Metchnikoff and phagocytosis theory. Nature Reviews Molecular cell Biology 2003; 4: 897–901.
Tauber AI, Chernyak L. Metchnikoff and the Origins of Immunology: From Metaphor to Theory. Oxford University Press 1991: 25–67.
Tauber AI, Chernyak L. The birth of immunology. II. Metchnikoff and his critics. Cell Immunol 1989 Jul; 121(2): 447–73.
Tauber AI. Moving beyond the immune self. Immunology 2000; 12: 241–8.
Podolsky S. Cultural divergence: Elie Metchnikoff's *Bacillus bulgaricus* therapy and his underlying concept of health. Bull History Med 1998; 72.1:1–27.
Burnet FM. The clonal selection theory of acquired immunity. Vanderbilt University Press 1959.
Jerne NK. Toward a network theory of the immune system. Ann Inst Pasteur Immunol (Paris) 1974; 125C: 373–89.
Barnes RD. Invertebrate Zoology. Third Edition. Philadelphia: WB Saunders, 1974.
Hood L, Huang HV, Dreyer WJ. The area code hypothesis. The immune system provides clues to understanding the genetic and molecular basis of cell recognition during development. J Systamol Struct 1987;7:531–559.
Thiery JP. Cell adhesion molecules as morphoregulators. A short survey. In: Burger MM, Sordat B, Zinkernagel RM, editors. Cell to Cell Interaction. Basel: Karger, 1990;242–246.
Aguayo AJ, Villegas-Perez MP, Vidal-Sanz M, Carter D, Bray GM. Influences of non-

neuronal tissues on the regeneration of injured nerve cells in the central nervous system of adult mammals. In Burger MM, Sordat B, Zinkernagel RM, editors. Cell to Cell Interaction. Basel: Karger, 1990; 40–61.
26. Sharon N. Lectin-carbohydrate complexes of plants and animals: An atomic view. Trends Biochem Sci 1993;18:221–226.
27. Berczi I, Szentivanyi A. Adhesion molecules. In "Neuroimmmune Biology", Volume 3: The Immune-Neuroendocrine Circuitry. History and Progress. Berczi I, Szentivanyi A, Editors, Elsevier, Amsterdam, 2003; pp. 99–115.
28. Murphy WJ, Reynolds CW, Tiberghien P, Longo DL. Natural killer cells and bone marrow transplantation J Natl Cancer Inst 1993;85:1475–82.
29. Haller O, Örn A. In vivo activity of murine NK cells. Editor Herberman RB. in Natural Cell-Mediated immunity Against Tumors, 1980; 1105–15.
30. Crocker PR, Varki A. Siglecs in the immune system. Immunology 2001;103(2):137.
31. Keissling R, Klein E, Wigzell H. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukaemia cells. Specificity and distribution according to genotype. Eur J Immunol 1975;5:112–7.
32. Herberman RB, Nunn ME. Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumours. I. Distribution of reactivity and specificity. Int J Cancer 1975;16:216–29.
33. Kärre K. How to recognize a foreign submarine. Immunol Rev. 1997;155:5–9.
34. Ezekowitz RAB, Hoffman JA. Innate Immunity. Totowa, NJ: Humana Press; 2003.
35. Gallucci S, Lolkema M, Matzinger P. Natural adjuvants: Endogenous activators of dendritic cells. Nat Med 1999; 5:1249–55.
36. Beutler B, Rietschel ET. Innate immune sensing and its roots: the story of endotoxin. Nature Reviews Immunology 2003; 3: 169–76.
37. Weber JR, Moreillon P, Tuomanen EI. Innate sensors for Gram-positive bacteria. Current Opin Immunol 2003; 15: 408–15.
38. Huttner, KM. Antimicrobial Peptides - The Defence Never Rests. In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 29–48.
39. Wong HR. Endogenous cytoprotective mechanisms. In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 49–65.
40. Bertok L, The Role of Bile Acids in Natural Resistance: physico-chemical host defence In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 67–78.
41. Herberman RB. A Historical Introduction of Natural Killer (NK) Cells and Current Status of Their Role in Host Defences In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 81–94.
42. Djeu JY, Blanchard DK. Regulation of human polymorphonuclear neutriphil (PMN) activity against Candida albicans by large granular lymphocytes via release of a PMN-activating factor J Immunol 1987;139:2761–2.
43. Lázár G, Husztik E, Lázár G, Jr. The Role of the Reticuloendothelial System in Natural Immunity In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 95–101.
44. Chow DA. Natural immune activation: stimulators/receptors. In Neuroimmmune

Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 123–150.
45. Arneson LN, Leibson PJ. Signalling in Natural Immunity: Natural Killer Cells In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 151–166.
46. Flo, TH, Aderem A. Pathogen recognition by Toll-like receptors In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 185–214.
47. Bosinger SE, Hoisawa KA, Cameron CM, Devries ME, Coombs JC. Cameron MJ, Kelvin DJ. Molecular control of leukocyte trafficking - Internal regulatory circuits of the immune system: Leukocyte circulation and homing In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004 (this volume).
48. Barlic J, Khandaker MH, Mahon E, Andrews J, DeVries ME, Mitchell GB et al. beta-arrestins regulate interleukin-8-induced CXCR1 internalization. J Biol Chem 1999;274(23):16287–94.
49. Barlic J, Andrews JD, Kelvin AA, Bosinger SE, DeVries ME, Xu L et al. Regulation of tyrosine kinase activation and granule release through beta-arrestin by CXCR1. Nat Immunol 2000;1(3):227–33.
50. Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 1998;393(6685): 595–9.
51. Poznansky MC, Olszak IT, Evans RH, Wang Z, Foxall RB, Olson DP et al. Thymocyte emigration is mediated by active movement away from stroma-derived factors. J Clin Invest 2002;109(8):1101–10.
52. Berczi I. Neuroendocrine Regulation of Natural Immunity. In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 215–262.
53. Pedersen BK. Natural immunity – Effect of exercise. In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 263–288.
54. Pedersen BK, Kappel M, Klokker M, Nielsen HB, Secher NH. The immune system during exposure to extreme physiologic conditions. Int J Sports Med 1994;15 Suppl 3:S116–21.
55. Droller MJ, Perlmann P, Schneider MU. Enhancement of natural and antibody-dependent lymphocyte cytotoxicity by drugs which inhibit prostaglandin production by tumor target cells. Cell Immunol 1978;39(1):154–64.
56. Hoffman-Goetz L, Simpson JR, Cipp N, Arumugam Y, Houston ME. Lymphocyte subset responses to repeated submaximal exercise in men. J Appl Physiol 1990;68(3):1069–74.
57. Bertok L. A new prospect for the enhancement of natural immunity. In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 289–307.
58. Larson SJ, Dunn A. Behavioral Mechanisms for Defense Against Pathogens In Neuroimmmune Biology, volume 5. Chow DA and Bertok L. Eds; Berczi I and Szentivanyi A. Series Eds; Amsterdam: Elsevier, 2004; pp. 351–368.
59. Medzhitov R, Janeway Jr CA. Decoding the patterns of self and nonself by the innate immune system. Science 2002; 296: 298–300.
60. Pilatte Y, Bignon J, Lambre CR. Sialic acids as important molecules in the regulation of the

immune system: pathophysiological implications of sialidases in immunity. Glycobiology 1993:3(3):201–18.
61. Elward K, Gasque P. Molecular Immunol. 2003; 40: 85–94.
62. Pangburn MK, Pangburn KL, Koistinen V, Meri S, Sharma AK. Molecular mechanisms of target recognition in an innate immune system: interactions among factor H, C3b, and target in the alternative pathway of human complement. J Immunol. 2000 May 1; 164(9): 4742–51.
63. Caucheteux SM, Kanellopoulos-Langevin C, Ojcius DM. At the innate frontiers between mother and fetus: linking abortion with complement activation. Immunity 2003; 18: 169–72.
64. Savill J, Fadok V. Corpse clearance defines the meaning of cell death. Nature 2000; 407: 784–8.
65. Ameisen JC. On the origin, evolution and nature of programmed cell death: a timeline of four billion years. Cell Death and Differentiation 2002; 9: 367–93.
66. Kerr JFR, Wylie AH, Currie AR. Apoptosis: a basic biological phenomenon with widespread implications in tissue kinetics. Brit J Cancer 1972; 26: 239–57.
67. Alenzi FQ, Warrens AN. Cellular and molecular themes in apoptosis. Wien Klin Wochenschr. 2003 Sep 15; 115(15–16): 563–74.
68. Lawen A. Apoptosis-an introduction. BioEssays 2003; 25: 888–96.
69. Sansonetti PJ. Phagocytosis, a cell biology view. J Cell Sci 2000;111(pt 19):3355–6.
70. Janeway CA Jr. The immune system evolved to discriminate infectious nonself from noninfectious self. Immunol Today 1992; 13(1):11–6.
71. Medzhitov R, Janeway CA Jr. Innate immunity: impact on the adaptive immune response. Curr Opin Immunol 1997;147(1):4–9.
72. Mushegian A, Medzhitov R. Evolutionary perspective on innate immune recognition. J Cell Biology 2001; 155: 705–10.
73. Stewart J. Immunoglobulins did not arise in evolution to fight infection. Immunol Today 1992; 13: 396–9.
74. Rinkevich B. Invertebrates versus vertebrates innate immunity: in the light of evolution. Scand J Immunol 1999; 50: 456–60.
75. Müller WEG, Blumbach B, Müller IM. Evolution of the innate and adaptive immune systems: relationships between potential immune molecules in the lowest metazoan phylum (porifera) and those in vertebrates. Transplantation 1999; 68(9): 1215–27.
76. Sunderkotter C, Steinbrink K, Goebeler M, Bhasdwaj R, Sorg C. Macrophages and angiogenesis. J Leuk Biol 1994; 55: 410–22.
77. Diez-Roux G, Lang RA. A macrophage induced apoptosis in normal cells *in vivo*. Development 1997; 124: 3633–38.
78. Diez-Roux G, Argilla M, Makarenkova H, Ko K, Lang R. Macrophages kill capillary cells in G phase of the cell cycle during programmed vascular regression. Development 1999; 126: 2141–7.
79. Veenstra van Nieuwenhovten AL, Heineman MJ, Faas MM. The immunology of successful pregnancy. Human Reproduction Update 2003; 9: 347–57.
80. Hunt JS, Petroff MG, Burnett TG. Uterine leukocytes: key players in pregnancy. Cell & Developmental Biology 2000; 11: 127–37.
81. O'Hagen DT, Valiente NM. Recent advances in the discovery and delivery of vaccine adjuvants. Nat Rev Drug Discov 2003;2:727–35.
82. Rogatsky I. Drug discovery for inflammatory diseases. Expert Opin Biol Ther 2003

Sep;3(6):1001–4.
83. Hackett CJ. Innate immune activation as a broad-spectrum biodefense strategy: Prospects and research challenges. J Allerg Clin Immunol 2003 ;112(4):686–94.
84. Hilleman MR. Overview: cause and prevention in biowarfare and bioterrorism. Vaccine 2002;20:3055–67.
85. Mercenier A, Pavan S, Pot B. Probiotics as biotherapeutic agents:present knowledge and future prospects. Curr Pharm Res 2003;9(2):175–91.
86. Tuohy KM, Probert HM, Smejkal CW, Gibson GR. Using probiotics to improve gut health. Drug Discov Today 2003;8:692–700.
87. Ouwehand AC. Probiotics: time to move beyond Metchnikoff? Drug Discov Today 2003 Dec;8(23):1063.
88. Butler RN. Economic and political implications of immunology and aging on tomorrow's society. Mech Ageing Dev 1997 Feb; 93(1–3):7–13.