Available online at www.sciencedirect.com
SCIENCE DIRECT®
Immunology Letters 93 (2004) 97–108
Immunology Letters
www.elsevier.com/locate/

Review

Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases

Helena Tlaskalová-Hogenová[a,*], Renata Štěpánková[a], Tomáš Hudcovic[a], Ludmila Tučková[a], Božena Cukrowska[b], Rája Lodinová-Žádníková[c], Hana Kozáková[a], Pavel Rossmann[a], Jiřina Bártová[d], Dan Sokol[a], David P. Funda[a], Dana Borovská[a], Zuzana Řeháková[a], Jiří Šinkora[a], Jaroslav Hofman[a], Pavel Drastich[e], Alena Kokešová[f]

[a] *Department of Immunology and Gnotobiology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic*
[b] *Department of Pathology, The Children's Memorial Health Institute, Aleja Dzieci Polskich 20, Warsaw, Poland*
[c] *Institute for Care of Mother and Child, Podolské nábřeží 157, Prague, Czech Republic*
[d] *Institute of Dental Research, Vinohradská 48, Prague, Czech Republic*
[e] *Institute of Clinical and Experimental Medicine, Vídeňská 800, Prague, Czech Republic*
[f] *Second Medical Faculty, Hospital Motol, Vúvalu 84, Prague, Czech Republic*

Received 29 May 2003; received in revised form 11 January 2004; accepted 18 February 2004

Available online 14 March 2004

Abstract

Commensal microflora (normal microflora, indigenous microbiota) consists of those micro-organisms, which are present on body surfaces covered by epithelial cells and are exposed to the external environment (gastrointestinal and respiratory tract, vagina, skin, etc.). The number of bacteria colonising mucosal and skin surfaces exceeds the number of cells forming human body. Commensal bacteria co-evolved with their hosts, however, under specific conditions they are able to overcome protective host responses and exert pathologic effects. Resident bacteria form complex ecosystems, whose diversity is enormous. The most abundant microflora is present in the distal parts of the gut; the majority of the intestinal bacteria are Gram-negative anaerobes. More than 50% of intestinal bacteria cannot be cultured by conventional microbiological techniques. Molecular biological methods help in analysing the structural and functional complexity of the microflora and in identifying its components. Resident microflora contains a number of components able to activate innate and adaptive immunity. Unlimited immune activation in response to signals from commensal bacteria could pose the risk of inflammation; immune responses to mucosal microbiota therefore require a precise regulatory control. The mucosal immune system has developed specialised regulatory, anti-inflammatory mechanisms for eliminating or tolerating non-dangerous, food and airborne antigens and commensal micro-organisms (oral, mucosal tolerance). However, at the same time the mucosal immune system must provide local defense mechanisms against environmental threats (e.g. invading pathogens). This important requirement is fulfilled by several mechanisms of mucosal immunity: strongly developed innate defense mechanisms ensuring appropriate function of the mucosal barrier, existence of unique types of lymphocytes and their products, transport of polymeric immunoglobulins through epithelial cells into secretions (sIgA) and migration and homing of cells originating from the mucosal organised tissues in mucosae and exocrine glands.

The important role of commensal bacteria in development of optimally functioning mucosal immune system was demonstrated in germ-free animals (using gnotobiological techniques). Involvement of commensal microflora and its components with strong immunoactivating properties (e.g. LPS, peptidoglycans, superantigens, bacterial DNA, Hsp) in etiopathogenetic mechanism of various complex, multifactorial and multigenic diseases, including inflammatory bowel diseases, periodontal disease, rheumatoid arthritis, atherosclerosis, allergy, multiorgan failure, colon cancer has been recently suggested. Animal models of human diseases reared in defined gnotobiotic conditions are helping to elucidate the aetiology of these frequent disorders. An improved understanding of commensal bacteria–host interactions employing germ-free animal models with selective colonisation strategies combined with modern molecular techniques could bring new insights into the mechanisms of mucosal immunity and also into pathogenetic mechanisms of several infectious, inflammatory, autoimmune and neoplastic diseases.

* Corresponding author. Tel.: +420-2-4106-2363; fax: +420-2-472-1143.
E-mail address: tlaskalo@biomed.cas.cz (H. Tlaskalová-Hogenová).

0165-2478/$ – see front matter
doi:10.1016/j.imlet.2004.02.005

Regulation of microflora composition (e.g. by probiotics and prebiotics) offers the possibility to influence the development of mucosal and systemic immunity but it can play a role also in prevention and treatment of some diseases.

Keywords: Mucosal microbiota; Intestinal barrier; Germ-free animal; Gut inflammation; Allergy; Autoimmunity; Probiotics

1. Introduction—basic characteristics and localisation of normal microflora

Each organism lives in a continuous interaction with its environment: this interaction is of vital importance but at the same time it could be life-threatening. The largest and most important interface between the organism and its environment is represented by surfaces covered by epithelial cells. Of these surfaces mucosae represent in humans about $300\,m^2$ while skin covers approximately $2\,m^2$ surface of the human body. Starting from first hours after the delivery from the sterile uterine environment (mammalian foetuses are born germ-free) the interaction of the macro-organism with micro-organisms begins: the main portal of entry of microbes is skin and mucosal surfaces of the gastrointestinal, respiratory and urogenital tracts. Physiologically occurring interaction with bacteria leads to colonisation of epithelial surfaces and this co-existence is usually harmonious, and beneficial for the host (commensalisms). A complex, open ecosystem, formed by resident bacteria and transiently present microbes interacting with macro-organism is founded. However, under some conditions the interaction with "endogenous" microbes can be harmful for the host (parasitism) and opportunistic infections can occur [1–3]. The microflora interacts with its host both locally and systemically. These interactions are characterised by active participation of both partners and the strategy of both of them seems to be similar: evolutionary co-existence has equipped both the micro-organisms and the immune system of the host with similar mechanisms of diversification and selection [4].

The number of autochthonous bacteria living on mucosal surfaces and skin exceeds the number of cells forming the human body. We, as human individuals, are thus complex ecosystems formed by normal microflora (approximately 10^{14} microbes) and by only 10^{13} mammalian cells! Normal microflora comprises mainly bacteria, but viruses, fungi and protozoa's are also present. Commensal bacteria exhibit enormous diversity; it is assumed that a minimum of 1000 species are involved. Our current knowledge about microbial ecosystems relies on known, cultivation-based techniques; however, the gut microflora consists mostly of anaerobic bacteria that are not easy to analyse by conventional culturing techniques [5]. Moreover, the description and identification of members of normal flora are hampered by the fact that many resident bacteria are non-cultivable (e.g. up to 80% of the intestinal microbial population might be uncultured!) [2,3,5,6]. Recently, molecular biological techniques are increasingly applied for studies of the complexity of resident microbial communities. Sequence analysis of clone libraries from amplified ribosomal DNA and denaturing or temperature-gradient gel electrophoresis have demonstrated the enormous diversity of species present in the gut. For quantitative analysis of gut flora, 16S ribosomal RNA-based oligonucleotides were designed that were applied as primers in PCR or as probes in fluorescent in situ hybridisation (FISH). For this purpose, probes covering about 80% of the total intestinal microflora has been already described [7–10].

Human oral cavity contains approximately 10^{10} bacteria (more than 500 bacterial species) inhabiting the teeth, gingival crevices, buccal mucosa and tongue. The tooth surface accumulates a complex of bacteria in aggregates or biofilms (bacteria forming these "dental plaques" are involved in dental carries). The main micro-organisms are streptococci and *Actinomyces* spp. Gingival crevices contain large numbers of Gram-negative anaerobes, among them bacteria assumed to be involved in the development of periodontal disease (*Porphyromonas gingivalis*) [1,9].

By inhalation of the air the respiratory tract comes into contact with large numbers of bacteria but the anatomical organisation and effective mechanisms (cleansing by ciliated epithelia, trapping by mucus) prevent microbial colonisation of distal parts of the respiratory tract. However, nose and nasopharynx contain staphylococci, streptococci, *Corynebacterium* and Gram-negative cocci. Interestingly, nasopharynx and oropharynx of a relatively high percentage of individuals contain also bacteria (so-called opportunistic pathogens) which can cause life-threatening infections like pneumonia, meningitis or less severe diseases like otitis, sinusitis, pharyngitis. Distal parts of the respiratory tract (trachea, bronchi and alveoli) are sterile [1,9].

In urogenital tract, the flushing by sterile urine ensures the sterility of the bladder and urethra, and only the distal part of female urethra is colonised by bacteria from anus, vagina or skin. Vagina is colonised, the composition of microbial community being dependent upon the hormonal state. Before puberty and after menopause, the prevailing bacteria are staphylococci and streptococci, after puberty estrogens cause a change (decrease) in the pH of vaginal secretions and lactobacilli become dominant.

Gastrointestinal tract is colonised by the large number of bacteria, mainly anaerobic. Due to the acidity of its contents and the presence of proteolytic enzymes, stomach represents a barrier preventing continuous access of bacteria from environment to the distal parts of the gastrointestinal tract. However, even in these conditions small numbers of bacteria are present; these are attached to gastric epithelia or present in mucus. Among them the presence of Gram-negative spiral-shaped *Helicobacter pylori* is of ut-

most importance. This organism causes gastritis and gastric and duodenal ulcers and is implicated in gastric cancer but it is present also in 30–80% of healthy individuals. The duodenum and jejunum have only a few bacteria, while ileum contains a massive and diverse microbial population (about 10^9 ml^{-1}). The highest numbers of bacteria displaying enormous diversity are found in colon where they are attached to the mucosa or are present in the contents. More than 90% of the bacterial population are obligate anaerobes, predominant species being: *Bacteroides*, *Eubacterium*, *Bifidobacterium*, *Fusobacterium*, *Peptostreptococcus* and others. Regularly present but in small proportion of the microflora are, e.g. *Escherichia coli*, *Enterobacter* and *Lactobacillus* [1,9].

Skin comes into a regular and frequent contact with bacteria from the environment, but the conditions for populating healthy skin are limited to such anatomical sites where moisture, temperature and presence of nutrients (swear and sebum) enable the survival of bacteria. The number of skin bacteria approaches 10^{12}, and this population includes mainly Gram-positive bacteria, obligate aerobes (*Micrococcus*) or facultative anaerobes (e.g. *Staphylococcus* and *Corynebacterium*) [1,9].

2. Defense mechanisms on mucosal surfaces

Epithelial surfaces are immediately after birth coming into contact with numbers of micro-organisms. These surfaces therefore evolved a number of protective mechanisms to resist the invasion of micro-organisms. While the skin surface is protected mechanically by several epithelial layers, surfaces of the gastrointestinal, respiratory and urogenital tracts, conjunctivae and outlets of endocrine glands are mostly covered with a single-layered epithelium and require, therefore, a more extensive protection: this is represented by a complex of mechanical and chemical agents responsible for effective degradation and removal of heterogeneous substances. In addition, both mucosa and internal environment of the organism are protected by a most effective innate and highly specific immune systems. Basic functions of the mucosal immune system are protection against pathogenic micro-organisms and prevention of penetration of immunogenic components from mucosal surfaces into the internal environment of the organism (barrier and anti-infectious functions). Another important function is induction of unresponsiveness of the systemic immunity to antigens present on mucosal surfaces (“oral, mucosal tolerance”) and maintenance of the homeostasis on mucosal surfaces (immunoregulatory function) [11,12].

Among the basic features of mucosal immunity differentiating it from systemic immunity, are strongly developed mechanisms of innate immunity and existence of characteristic populations of lymphocytes that differ from, e.g. blood lymphocytes in origin, phenotype and secreted products. Other features characteristic of mucosal immunity are: colonisation of mucosal surfaces and exocrine glands by cells originating from lymphatic follicles of intestine or bronchi (migration and homing of mucosal lymphocytes establishing the so-called “common mucosal system”) and the well-known epithelial transport of polymeric immunoglobulins produced by mucosal plasma cells through the epithelium (secretory immunoglobulins, mainly IgA isotype) [11–14].

A balance in intestinal mucosa may be disturbed by pathogenic micro-organisms and their toxins, or by inadequately functioning components of the mucosal immune system [14,15]. On the other hand, an expression of pathologically increased immunological activity may induce various inflammatory processes. Thus, numerous chronic diseases may occur as a result of disturbances of mucosal barrier function or of changes in mechanisms regulating mucosal immunity. This may involve infectious diseases, inflammatory diseases (allergies), multiorgan failure but also autoimmune diseases developing either in their initial phase or throughout on mucosal surfaces.

3. Barrier function of mucosae, innate immunity, epithelial cells

The main mechanical barrier of mucosal surfaces is formed by a layer of epithelial cells covered with glycocalyx composed of complex glycoproteins. The epithelium of most mucosal surfaces consists of a layer of interconnected, polarised epithelial cells separated by a basal membrane from the connective and supporting tissue surrounding various types of cells present in the lamina propria. Intestinal epithelial layer is reinforced by tight junctions present in paracellular spaces of epithelial cells and forming an interconnected network. Tight junctions were found to act as a dynamic and strictly regulated port of entry that opens and closes in response to various signals (e.g. cytokines) originating in the lumen, lamina propria and epithelium. Tight junctions participate in preserving cellular polarity and are regarded as key elements in intestinal diffusion mechanisms. The molecules forming tight junctions (zonulins, occludins, claudins) are connected to the cytoskeleton of epithelial cells [16,17]. In addition to laterally situated tight junctions present in *zonula occludens*, there are intermediary junctions and desmosomes in the so-called *zonula adherens*.

Various types of epithelial cells participate in mucosal barrier function: the main and most frequent cells are conventional enterocytes (colonocytes in colon), of importance are goblet cells producing both mucus and trefoil peptides required for epithelial growth and repair, enteroendocrine cells produce neuroendocrine molecules having a paracrine effect, and Paneth cells secreting antibiotic peptides-defensins. Epithelial cells are maintained on a network of interconnected myofibroblasts, which produce molecules necessary for the basal membrane in addition to factors required for epithelial growth. Mucosal barrier function is greatly influenced by the products of the nervous system (neurotransmitters), increas-

ing, for instance, the passage of macromolecules through tight junctions. It has been described that nervous signals emitted under stress are transmitted directly to mucosal mast cells and deposited in the lamina propria under the basal membrane, where they are in direct contact with nerve endings [11,18].

A basic mechanism of mucosal immunity is innate, natural immunity represented by processes that protect the host immediately, within the first minutes and hours, of exposure to infection. It is of interest that these defense mechanisms of vertebrates are implemented by structurally related effector molecules present in plants and insects, which do not possesses higher, specialised forms of adaptive immunity. A characteristic, although not yet clearly defined, feature of innate immunity is an ability of distinguishing between potentially pathogenic microbial components and harmless antigens by "pattern recognition receptors (PRRs)". An example of these molecules is the so-called Toll-like receptors (TLRs) enabling mammalian cells to recognise conserved characteristic molecules present on microorganisms and described as pathogen-associated molecular patterns (PAMP) [19–21]. As these molecules e.g. lipopolysaccharides, peptidoglycans and others are present also on commensal bacteria it seems more precise to call them microbe-associated molecular pattern (MAMP). Toll receptors were originally described in *Drosophila* as transmembrane receptors, their extracellular domain contain leucine-rich repeat whereas cytoplasmic domain is homologous to IL-1R, in insects they were found to play an essential role in the immune response to fungal infection. In mammals, PRRs are present on macrophages, neutrophils, dendritic cells and other cells belonging to innate immune system. It was demonstrated that recognition of microbes activates NfκB signalling pathway and in this way it triggers cytokine production, up-regulation of co-stimulatory molecules on antigen presenting cells leading to activation of T cells. Innate immunity is closely linked to adaptive, acquired immunity [19,21].

Under physiological conditions the gut, i.e. the largest body surface, is realising complex and poorly understood cell interactions which regulate responses to food antigens and to antigens of the normal (commensal) bacterial flora in close proximity to a large population of rapidly renewing epithelial cells, specialised intraepithelial lymphocytes and other immunologically active cells present in the mucosa. Cells of innate immunity produce cytokines essential for inflammatory reactions as well as factors critical for the subsequent initiation of specific immunity. It is the contact with bacteria and their components through the sensing structures (PRRs) on their surface that initiate innate immunity responses [21].

TLRs share high homology in the signal transduction structures and pathways with a particular class of cytokine receptors, i.e. the receptors for IL-1 and IL-18, two cytokines involved in the non-specific amplification of immune responses. Very interesting and important is the expression and modulation of TLR/IL-1R on dendritic cells which represent the link between innate and adaptive immunity and on epithelial cells which play an active role during interaction with external environment and in regulating the mucosal immune responses. The role of other molecules which are able to bind bacteria and their components, e.g. CD14, LPS-binding protein (LBP) and others is in focusing bacteria and bacterial products to TLR, thereby modulating their activation.

Epithelial cells are an integral and perhaps the most important part of a innate, natural defense mechanism of mucosal surfaces. It has been recently found that intestinal epithelial cells as an important part of the innate immune system are directly involved in various immune processes, in addition to their absorptive, digestive and secretory functions. Their capacity of transporting secretory immunoglobulins produced by plasma cells in lamina propria to the lumen by binding these to a receptor of polymeric immunoglobulin (pIgR) was described [22]. There is strong evidence that epithelial cells can present antigens [23]. Epithelial cell express numerous molecules that are involved in antigen presentation: transplantation antigens class I both classic and non-classic (HLQ A–C, HLA E, CD1d, MICA/MICB), transplantation antigens class II.

Immunologically active cells of mucosal surfaces (T and B cells, macrophages, neutrophils, eosinophils, mast cells) produce mediators influencing epithelial physiology and its various functions (barrier, transportation). Epithelial cells were found to react to proinflammatory cytokines either by the synthesis or production of immunologically active proteins. Incubation of epithelial cells with interferon-γ or TNF is followed by increased expression of a secretory component—receptor for polymeric immunoglobulins (pIgR), class II transplantation antigens, pattern recognition receptors and other surface molecules. Cells of epithelial lines were found to produce constitutively or by induction number of cytokines, chemokines and mediators [11,12,22,23].

Epithelial cells have been found to perform various immunological functions, which involve interaction with other cells of the immune system and an inflammatory response to microbial invasion. We have shown that human intestinal epithelial cells express an important lipopolysaccharide-binding molecule CD14 which together with Toll-like receptors can participate in maintaining the intricate balance between the self and outer environment in the gut [24]. Furthermore, we found that these cells release soluble form of CD14 which may have important implication in shaping the interaction between the mucosal immune system and gut bacteria. Recently, the molecular consequences of the interaction of components of commensal microflora with macroorganism has been characterised by a new approach, i.e. by analysing the transcriptional response after colonisation of the gut of adult germ-free mice [10,25,26].

In addition to well-known humoural components of innate immunity (humoural forms of PRRs) present on mu-

cosal surfaces such as complement, lysozyme, lactoferrin, mannan-binding protein and others, new, recently described factors have been the subject of intensive study. An important component of non-specific mechanisms are antimicrobial peptides widely distributed throughout plant and animal kingdom. Various defensins and one type of cathelicidins were found in intestinal epithelial cells. α-Defensins are present in apical granules of Paneth epithelial cells, β-defensins are found in airway, gingival, gastrointestinal mucosa and in salivary glands. In addition to their wide antimicrobial activity, defensins display chemotactic activity toward dendritic cells and T cells, in other words, they represent components of innate immunity interacting with adaptive immunity [27].

4. Mucosa-associated lymphatic tissues (MALT), common mucosal immune system, oral (mucosal) tolerance

Mucosa-associated lymphatic tissue consists partly of organised tissue representing both solitary and multiple lymphatic follicles (Peyer's patches, appendix) and freely dispersed lamina propria lymphocytes (LPL). These components in co-operation with components of innate mucosal immune system, accomplish specific (adaptive) immune responses. Organised lymphatic tissue (Peyer's patches and lymphatic follicles) represents an inductive site of the mucosal immune system. Germinal centres of lymphatic follicles consist mainly of differentiating B cells, T lymphocytes occupy interfollicular space preferably around venules with a high endothelium. Organised lymphatic tissue is covered with an epithelial layer (follicle-associated epithelium (FAE)) containing a special type of membranous epithelial cells called "M" cells. Specialised M cells are most effective in absorbing particular antigens and transporting these from lumen to follicular environment (Peyer's patches), where these antigens activate T lymphocytes and induce thus mucosal immunity [11,13]. Diffuse lymphocytes represent an efficient effector component of the mucosal immune system. Lymphocytes are present in the epithelium on the basolateral side of enterocytes—these are called intraepithelial lymphocytes (IEL)—T cells, predominantly CD8+ in nature, differ from cells present in the bloodstream. Intraepithelial lymphocytes have several features in common: phenotype CD8+, CD45RO+, adhesive molecules (integrin αEβ7) and cytoplasmatic granules containing cytolytic proteins (perforin). It was suggested that intraepithelial lymphocytes may be capable of identifying proteins that generally are not present in the epithelium and to react cytolytically to damaged or changed epithelial cells. Diffuse lymphocytes of lamina propria are the most numerous and the most active mucosal effector cells. They are represented by T cells, predominantly CD4, and B cells producing polymeric IgA. Mucosal T cells produce various cytokines, an important and interesting population of regulatory T cells is noted mainly for its suppressive activity. These regulatory T cells express CD25 and were shown to produce inhibitory cytokines IL-10 and TGFβ [11,12,14,28,29,30].

IgA is one of the most important humoural defense factors on mucosal surfaces. Its polymeric form dominates in secretions, monomeric form in circulation. The main source of monomeric serum IgA is bone marrow, of polymeric IgA plasma cells in mucosa and exocrine glands. The gut-associated lymphatic tissue (GALT) is the largest lymphatic organ of the body. Total amount of IgA-producing cells of the intestine (7×10^{11}), and the daily IgA production in intestine (2–5 g) indicate that IgA is the most represented class of the immunoglobulins in the body. Most of IgA specifities are directed against mucosal microbiota. The molecular structure of polymeric IgA enables this molecule to penetrate into secretions, resist enzymatic activities and function as an effector molecule on mucosal surfaces [11,12,31–34]. Secretory IgA, i.e. dimeric IgA molecule, is resistant to proteolysis and its primary task is to prevent both adherence of bacteria to mucosal surfaces and penetration of antigens to the internal environment of the organism. Moreover, IgA is capable of reacting with several non-specific bactericidal substances present in secretions (e.g. lactoperoxidase, lactoferrin) and transport these to bacterial surfaces [33–35].

Migration of lymphocytes from inductive parts of mucosal immune system and their homing in effector parts of mucosae and exocrine glands ("common mucosal immune system") is a good example of a multiphasic process of interaction between lymphocytes and their original environment. It was confirmed that this selective homing of lymphocytes to epithelial surfaces concerns mainly recirculating, activated, blastic forms of lymphocytes and small, memory lymphocytes. These lymphocytes (both of man and mouse) have a characteristic α4β7 integrin on their surface and act as receptor of specific mucosal venous adresin MadCAM/1 present on endothelial cells of mucosal capillaries. A good example of the effect of migration and selective colonisation by cells from intestinal mucosa is offered by the composition of mammary gland secretion—maternal milk. Apart from nutritive components, mother's milk contains a number of immunologically non-specific and specific factors and a large quantity of immune cells: all these milk components protect the not yet completely mature intestine of the infant. A consequence of a colonisation of the mammary gland by cells from the intestine is a presence of IgA antibody and of cells directed against antigens present in maternal gut ("enteromammary axis"), i.e. mainly bacteria belonging to maternal microflora that colonise the intestine of the infant within the first days of life [11,12,14].

The food contains a complex mixture of plant and animal products which are immunogenic. Part of these antigens (1–2%) is absorbed through gut mucosa into the circulation in intact, i.e. immunogenic form. Oral application of antigens leads to the development of inhibition of systemic immune response to parenterally administered antigens ("oral tolerance"). Individual mucosae seem to differ in their abil-

ity to induce mucosal tolerance; intestinal and nasal mucosae belong to the most sensitive sites of tolerance induction. Oral tolerance to food antigens and to commensal flora is installed during the first weeks after birth. Although the mechanism of oral or mucosal tolerance needs to be clarified, there is good reason to believe that, depending on the dose of antigen, two mechanisms are involved: deletion and anergy associated with a high dose of antigen and induction of active suppression mediated by regulatory T cells occurring with a low dose. The mechanism of suppression (oral tolerance) is caused by the presence of inhibitory lymphokines, mainly TGFβ and IL-10, produced by regulatory T cells (the so-called Tr or Th3 cells) [11,14,30,36].

5. Immunological and physiological functions of normal flora: studies in germ-free animals (contribution of gnotobiology)

Commensal microflora is an integral part of a complex of natural mechanisms on mucosal surfaces and skin that safeguard the resistance of the organism against pathogenic micro-organisms. At an optimal composition, it prevents attachment and multiplication of pathogenic micro-organisms on these surfaces ("colonisation resistance") and their invasion into epithelial cells and circulation. Intestinal microflora plays an important role in anti-infectious resistance both by direct interaction with pathogenic bacteria and by its influence on immune system. Components of intestinal microflora play a crucial role in postnatal development of the immune system; during the early postnatal period intestinal microflora stimulates the development of both local and systemic immunity. Later on, these components evoke, on the contrary, regulatory (inhibitory) mechanisms intended to keep both mucosal and systemic immunity in balance [9,14].

Invaluable tool for studying the role of normal microflora and for distinguishing genetically determined spontaneously developing immune mechanisms from those induced by environmental agents is represented by animal models reared in germ-free conditions [37–45]. Gnotobiological techniques developed since the end of 19th century made it possible to establish various germ-free models: usually, the first generation of animal species or strain is born by caesarean section, transferred into isolators for germ-free rearing and foster mother-fed or hand-fed by cow milk-based formulas. The composition of the diet must take into consideration the lack of bacterial activities connected with nutrition. The best defined and immunologically purest gnotobiological model is an antigen-free animal lacking both microflora and food antigens. However, antigen-free models are used rather exceptionally because chemically defined non-antigenic diet for feeding of germ-free animals is difficult to prepare [40,45]. Germ-free rodents but also chickens, calves, pigs and goats were most often used. Alterations of intestinal morphology, changes in GALT structure and functions were described in all animals reared under germ-free conditions. The most pronounced differences were described in the large intestine, e.g. accumulation of mucus with retention of water as a consequence of missing mucolytic bacteria occurs in germ-free rodents and caecum is much larger (up to 10 times) than in conventionally reared animals. Prolonged intestinal epithelial cell cycle in germ-free mice (4 days) when compared with conventionally reared mice (2 days) and decrease in peristalsis were described. Intestinal lymphatic constituents are underdeveloped in germ-free animals, numbers of lymphocytes present in organised and diffuse lymphatic gut-associated tissues are decreased [40,42,43]. Microbial deprivations was shown to influence preferentially the α/β bearing TCR intraepithelial lymphocytes [46].

We and others have shown that intestinal colonisation of germ-free animals with defined commensal microbes has important effects on mucosal and systemic immunity. It results in an increase of immunoglobulin level, production of natural antibodies and specific antibodies against colonising bacteria, and an increase of the overall immunological capacity [43]. We found that migration pattern of lymphocytes into mucosal sites of rats was influenced by the presence of microflora [43,44]. In pigs, the transport of maternal antibodies does not occur due to the unique structure of porcine placenta (six layers) and new-borns transferred into germ-free conditions are deprived of colostrum and represent thus an "immunologically virgin" model. We have shown that rearing of new-born pigs, rats and rabbits in germ-free environment profoundly delays the appearance of cells spontaneously secreting natural antibodies against bacterial antigens (LPS) and tissue antigens detected mainly in mucosal lymphatic tissues (rabbit appendix, Peyer's patches of rats, ileal patches of pigs) [42,43]. By comparing the antibody responses and others we have shown that germ-free animals in comparison with colonised animals have less diversified immunoglobulins [43,47–51]. Lymphocytes from germ-free animals reacted to mitogens and polyclonal stimuli with low intensity. The development of isotype and specific antibody repertoire in germ-free pigs demonstrated the pronounced impact of intestinal colonisation on development and activation of the whole immune system. Interestingly, B cells producing spontaneously Igs in thymus were not influenced by microbial colonisation. In contrast to germ-free counterparts, heavy cellular infiltration of gut mucosa, formation of germinal centres in Peyer's patches, transient translocation of bacteria into mesenteric lymphnodes, production of specific antibacterial antibodies and polyclonal B cell activation with production of immunoglobulins were demonstrated during the first days after monocontamination of new-born germ-free pigs with a non-pathogenic *E. coli* strain [47,52]. Interestingly, after 5–10 days lasting activation of the pig and human immune system the local and systemic responses are inhibited (induction of oral tolerance to microflora?) [51–53]. Components of normal microflora thus induce in the gut "physiological" inflammatory response followed by balanced and controlled responses (self-limiting response) [41,42,47,52,53]. The physiologically occurring inflamma-

tory response in the gut during early postnatal ontogeny is thus essential for the development of the immune system and for its appropriate functioning [42,45].

The participation of the innate immunity in the interaction of commensal micro-organisms with the host immune system was studied at the beginning of gnotobiotic research. Phagocytic activity and macrophage chemotaxis were found decreased in germ-free animals. We have demonstrated differences in the response to lipopolysaccharide between germ-free and conventional animals. However, most of innate components begin to be identified, characterised and compared in germ-free and conventional animals. Recently, new interesting class of angiogenic proteins with microbicidal activity induced by colonisation with *Bacteroides thetaiotamicron* was described [54].

The metabolic potential of intestinal microflora as regards the number of biochemical reactions is similar to the metabolic potential of the liver. Bacteria play an important role in numerous metabolic processes of the intestine such as fermentation of dietary residues and mucus (saccharides and proteins) with generation of short-chain fatty acids, metabolism of xenobiotics, activation or destruction of mutagenic metabolites and vitamin synthesis [9,55]. Comparative studies of the possible microbial influences on enzymatic activities of the brush border membranes in small intestinal mucosa using germ-free and conventional mice and rats have been performed [56]. Differences in the activity of enterocyte brush border enzymes between germ-free and conventional animals were not apparent until after weaning when the germ-free animals showed a higher activity than the conventionally reared counterparts. Administration of caecal contents from conventional animals led to a decrease of enzymic activities to normal conventional levels. Monoassociated animals often show gut mucosal morphology and enterocyte enzymic activities half-way between germ-free and conventional animals. Not only the activities of the enzymic glycoproteins of microvillar membrane, but also the synthesis of sugar chains of membrane-associated glycoproteins were affected by the introduction of microorganisms. The effects of the microflora started to be studied by current methods of functional genomics [6,25].

6. Components of commensal bacteria with strong effects on innate and/or adaptive immunity

There is no doubt that not only living and multiplying bacteria but also their components which are expressed, secreted and could be released from bacterial body after microbial death are responsible for various immunomodulatory effects. Only some of them could be mentioned.

6.1. Lipopolysaccharides (endotoxins)

Bacterial lipopolysaccharides (LPS) are major outer surface membrane components present in almost all Gram-negative bacteria, which act as extremely strong stimulators of the innate immune system. They can cause septic shock in mammals including man. LPS consist of a hydrophobic domain known as lipid A, a non-repeating “core” oligosaccharide, and a distal polysaccharide (or O-antigen). Lipid A, the hydrophobic anchor of LPS, is a glucosamine-based phospholipid that makes up the outer monolayer of the outer membranes of most Gram-negative bacteria. The existence of a receptor-binding lipopolysaccharide has been the subject of intense debate during the past years, which has been fuelled by the discovery of lipopolysaccharide-binding protein (LBP) that interacts with the CD14 molecule expressed by monocytes and macrophages. In addition, the importance of more peripheral endotoxin-activated mediator systems, including the contact system, the tissue factor pathway, the fibrinolytic system, the complement system and nitric oxide for local and generalised inflammation has been unravelled in detail in the past years [3,57,58].

Much of the sequelae associated with endotoxin effects result primarily from excessive production of mediators such as cytokines tumour necrosis factor alpha (TNFα) and interleukin-1β by LPS-activated macrophages and monocytes. These, in addition to anaphylatoxin C5a of the complement system and interferon-γ, play a major role in the activation of different plasma cascade systems and in amplifying the release of soluble mediators, respectively [3,57,58]. Within minutes of recognising LPS, an array of cell types expressing relevant PRR (CD14 and TLR4) initiate defensive actions that mediate protection against the micro-organism, including the production of reactive oxygen intermediates and secretion of inflammatory cytokines. Cytokines initiate a cascade of signals to cells of the adaptive immune response, preparing them for the development of antigen-specific immune responses. Endotoxin exposure also results in production of defensins, which comprise several distinct families of antibacterial, antifungal and antiviral peptides [3,19,57,58].

6.2. Peptidoglycans (PGN)

PGN is an essential component of cell wall of virtually all bacteria, especially abundant is in cell wall of Gram-positive bacteria where other polysaccharides and proteins are covalently bound to it. In Gram-negative bacteria PGN is located under the LPS containing outer membrane. PGN surrounds the cytoplasmic membrane in bacteria and maintains their shape. PGN is polymer formed by β(1–4)-linked *N*-acetylglucosamine and *N*-acetylmuramic acid cross-linked by peptides. PGN is recognised by eukaryotic innate immune system. Several PGN recognition molecules including CD14, TLR2, family of peptidoglycan recognition proteins (PGRPs), cytoplasmic proteins Nod1 and Nod2 and PGN-lytic enzymes (lysozyme and amidases) are able to induce various host responses or have direct antimicrobial effects [59].

6.3. Bacterial CpG–DNA motifs

In contrast to the long-known effects of LPS and PGN the immunostimulatory properties of bacterial DNA were described only in the last decade. Soon after its discovery the stimulatory property was connected with single-stranded oligodeoxyribonucleotides (ODN). The crucial structure was shown to be connected with unmethylated cytosine–guanosine (CG) core. The lack of stimulatory activity of mammalian DNA results from structural differences from bacterial DNA: it contains only a few CG–dinucleotide motifs which are unmethylated. The optimal stimulatory sequences for the preparation of synthetic immunostimulatory ODN (about 20 nucleotides in length, the biological effect being affected also by regions adjacent to CG) were determined. Immunostimulatory DNA containing CpG motifs (CpG–DNA) was shown to stimulate mainly cells of the innate immune system, particularly dendritic cells and macrophages. Like LPS, CpG–DNA stimulates the production of a similar array of cytokines and expression of maturation markers. The crucial involvement of TLR9 in recognition of bacterial DNA was recently demonstrated [3,60,61].

6.4. Heat shock proteins (Hsps)

Hsps were first described by researchers studying the effects of heat stress. High temperature was shown to induce the production of proteins which were termed heat shock proteins or stress proteins. These proteins occur constitutively in all types of cells (bacterial, plant, etc.), they constitute about 5% of the cellular proteins and their function is to catalyse the correct folding of proteins (molecular chaperones). When the cells are exposed to stress protein denaturation occurs and the synthesis of Hsps is consequently triggered, their levels may rise to 15–20%. A characteristic feature of Hsps is their significant structural homology; for example, bacterial and human Hsp 60 share about 50% positional identity in their amino acid sequences. Hsps have been implicated in autoimmunity as up-regulated targets of adaptive immunity during inflammatory stress but recently also as triggering factors for innate immunity through activation via TLRs (TLR2 and TLR4). The analysis of T cell responses has shown that bacterial Hsp can trigger self-Hsp cross reactive response and that these responding T cells may inhibit autoimmune reactions [3,9,62].

6.5. Superantigens (SAG)

SAG represent a family of proteins stimulating potent immune responses directly by acting similarly as mitogens. T cell superantigens are bound without preceding processing to class II histocompatibility complex molecules expressed on antigen presenting cells and presented to T cells which recognise them by means of some types of the T cell receptor β chains. The selective binding of Vβ chains of the T cell receptor explains the high frequency of T cells responding to SAG (activation of up to 20% of T cell population) [3,9]. Some superantigens stimulate B cells via T cell mediated induction of polyclonal activation of B cells, considerable portion of microbial components is bound directly to a certain site in defined (domains) of surface immunoglobulins and belong therefore to the B cell superantigens [63]. Superantigens secreted mostly by Gram-positive bacteria (e.g. *Staphylococcus aureus* and *Streptococcus pyogenes*) are capable of inducing acute pathological symptoms, but they can also take part in initiating and maintaining chronic diseases [64]. Interestingly their involvement in allergy (atopic dermatitis, allergic rhinitis) has been recently demonstrated [65].

7. Mechanisms by which mucosal surfaces resist to proinflammatory effects of normal microflora components—microbe-associated molecular patterns

Like other bacteria commensals express MAMPs and produce exotoxins. Bacteria have short life span, significant cell death occurs in bacterial population followed by the release of wide range of bacterial components including DNA. One of the basic, intriguing but yet unsolved questions is why commensal bacteria do not trigger (in contrast to pathogens) inflammatory responses in mucosal tissues of the normal, healthy host [3].

The explanation of this paradox lies most probably in the peculiarity of mucosal immunity mechanisms. The entire cell which is in first contact with commensal bacteria is epithelial cell. One possibility is that epithelial cells depending on their localisation may be relatively unresponsive or they can be programmed for down-regulation of proinflammatory responses. It was shown that expression of some pattern recognition receptors required for LPS and other microbial components is lacking or lower in contrast with other cells belonging to the innate immune system (e.g. TLR4 expression) [66,67].

In vivo findings support the idea of low expression of PRRs in the cells of healthy gut: it was shown that intestinal macrophages do not express CD14, but under inflammatory conditions the reappearance of this marker on intestinal macrophages was demonstrated [68]. Recently, the presence of TLRs on regulatory T cells exerting immunosuppressive activity was described [69]. Commensals can down-regulate epithelial proinflammatory responses to their components; interference with signalling by inhibition of IκB ubiquitination occurring in epithelial cells cultured with commensal bacteria but not with pathogenic *Salmonella* was recently demonstrated [70]. However, the participation of other kinds of intestinal cells of innate and adaptive immunity (macrophages, NK cells, T and B lymphocytes) in down-regulatory processes seems to play important role [14]. The alternative possibility is exclusion of commensal bacteria from the luminal site of epithelial cells (the bacteria are not

allowed to penetrate through the mucus layers) and their binding to epithelial cell surface. The key role of mucosal dendritic cells in distinguishing pathogens from luminal microflora was recently suggested [71].

Finally, it is not excluded that the failure to produce inflammatory cytokines in mucosal cells is caused by the ability of commensal bacteria to produce proteins with immunosuppressive effects and/or that expression of MAMPs on commensal bacteria is lower. Gut bacteria are mainly anaerobic, not easy to cultivate, most of them are not yet identified or well characterised and their MAMPs and other products have usually not been analysed. It is possible that commensal bacteria similarly as viruses produce proteins (bacteriokines) which are able to inhibit the synthesis or action of host cytokines. Similarly as in the case of Arg-1 gingicain proteinase of *P. gingivalis* which cleaves and inactivate cytokines IL-1 and IL-6 there is now growing evidence that commensal bacteria can modulate inflammatory mechanisms by their released proteinases [3,9]. It is not excluded that proteins produced by commensal bacteria and modulating mucosal cytokine networks could be used for new anti-inflammatory therapeutic strategy [72].

8. Involvement of infectious components present on mucosal surfaces in aetiology and pathogenetic mechanisms of idiopathic, inflammatory and autoimmune diseases

The main characteristics of inflammatory and autoimmune diseases are tissue destruction and functional impairment as a consequence of immunologically mediated mechanisms which are principally the same as those functioning against dangerous (pathogenic) infections. In case of autoimmune diseases, a major effort was done in understanding pathogenetic mechanisms leading to the loss of tolerance to self components (autoantigens) [73,74]. Despite the fact that target antigens and the genetic basis of several autoimmune diseases are now better understood, the initial events leading to a loss of tolerance towards self-components remain unknown. One of the most attractive explanations for autoimmune phenomena has always centred on various infections as possible natural events capable of initiating the process in genetically predisposed individuals [14,44,74,75]. Increased interest in infectious agents as causes of chronic diseases was awakened by the discovery of *H. pylori* as a causative agent of stomach ulcer, chronic gastritis and probably also of gastric cancer.

A number of defined micro-organisms have been shown to evoke autoimmunity. Infection with intestinal microbial pathogens such as *Salmonella*, *Shigella* and *Yersinia* can trigger autoimmune reactions in joints and other organs [76]. Diseases with autoimmune features such as rheumatic fever and acute glomerulonephritis may develop after a streptococcal infection. Also, viral infections can bring about autoimmune reactions: for instance an infection with coxsackie virus is accompanied by a severe autoimmune myocarditis.

The most accepted hypothesis explaining how infectious components cause autoimmunity is based on the concept of cross-reactivity, “molecular mimicry”. This hypothesis assumes a similarity between the epitopes of an autoantigen present in the afflicted organism and the epitopes in the environmental antigen. The latter may consist of a micro-organism or an other external antigen that causes the autoimmune response [77]. Moreover, in identical twins, autoimmune disease does not necessarily develop in both subjects. Using specific T cell clones and a broad spectrum of peptides derived from the basic myelin protein it was demonstrated that the activation of autoaggressive cells can be the consequence of a binding of structurally related but not necessarily identical peptides [78]. From this finding, one can conclude that the stimulation of specific autoreactive cells can take place following a binding of structurally similar peptides originating from different environmental sources, viral, bacterial and food. Sequentially appearing responses to autoantigen epitopes or autoantigenic molecules (epitope spreading) is a characteristic feature described in developing autoimmune diseases. “Bystander” activation of immune cells was recently shown as another mechanism by which autoimmune reactivity could spread.

Infectious stimuli may participate in the development of autoimmune conditions by inadequate activation of components of the innate immune system. Antigen presenting cells, mainly dendritic cells performing the interaction of innate and adaptive immunity, could be activated (leading to maturation) by microbial components (MAMPs) through PRRs expressed on dendritic cells. Adjuvant activity of microbial components or their synthetic substitutes is described to correspond to the degree of activation of dendritic cells. During activation of dendritic cells, expression of co-stimulatory molecules increases which can lead to changes in the presentation of self antigens. Increased synthesis and expression of stress proteins (Hsp), chaperones and transplantation antigens leads to abnormal processing and presentation of self antigens by changing the transport and processing of intracellular peptides. Abnormal presentation of antigens can then evoke a response to cryptic self epitopes equal to the response to a dominant autoantigen [15,45,62,63,77,78]. Superantigens are considered to be one of the most effective bacterial components to induce inflammatory reactions, they are believed to take part in the induction and course of autoimmune mechanisms [64,65].

Dysregulation of the intestinal immune response to normal bacterial flora was suggested to play a crucial role in several inflammatory and autoimmune diseases [22,79,80,81]. Experimental animal models of human diseases (e.g. models of human inflammatory bowel disease, rheumatic diseases) reared in germ-free conditions did not develop the diseases and thus confirmed the important the role of normal flora [30,45,82,83]. The potential members of microflora responsible in inflammatory bowel disease (IBD) and other diseases

are currently analysed by using intentional colonisation of germ-free animals with defined bacterial strains [30,45,82]. However, in some cases the role of food antigens in development of autoimmune diseases is not excluded [84].

Genetically based or environmentally induced changes in mechanisms regulating mucosal immunity and tolerance can lead to impaired mucosal barrier function, increased penetration of microbial components into the circulation and consequently to exaggeration of aberrant immune responses and inflammation. In fact, increased permeability of the gut mucosa was demonstrated in patients with some autoimmune diseases and in their relatives [85].

9. Probiotics

Recent increased interest in an influence of intestinal microflora on human and animal health resulted in attempts of improving optimally its composition by using probiotics (most frequently bacteria of lactic fermentation and probiotics) [80,83,86]. Probiotics are defined as live cultures of micro-organisms administered orally and acting beneficially on host health. They influence favourably both development and stability of the microflora, inhibit colonisation by pathogens, influence the mucosal barrier by their trophic effect on intestinal epithelium and stimulate both specific and non-specific components of the immune system, moreover, they may well replace antibiotics whose resistance is steadily increasing [83,87]. After genetic manipulation, the commensal and probiotic strains could be used as vectors for clinically important molecules and for their preventive and therapeutic mucosal application [88]. Orally administered probiotic bacteria are expected to survive passage through stomach and colonise intestinal mucosal surfaces even if it were only for a short period. In addition to bacterial strains *Lactobacillus* and *Bifidobacter*, also some non-pathogenic strains of *E. coli* proved suitable for this purpose. We have shown that colonisation of new-borns with a non-pathogenic strain of *E. coli* stimulated local and systemic humoural and cellular immune responses and reduced the number of pathogens [53,89]. Recently, we have found that repeated oral application of non-pathogenic *E. coli* strain in the early postnatal period prevented the incidence of allergies as confirmed by a long-term (10 and 20 years) study [90]. Understanding the regulation of mucosal immune responses to commensal microflora maybe the key to targeted manipulation of microflora composition and successful intervention in wide range of chronic diseases.

Acknowledgements

The work was supported by grants No. 310/01/0933, 310/02/1470 (Grant Agency of the Czech Republic), grants No. A 5020101, A 5020205, A 5020210, S 5020203 (Grant Agency of Czech Academy of Sciences), grants No. NK 6742-3, NI 7525-3 (Internal Grant Agency of Ministry of Health of the Czech Republic) and AVOZ 5020903 (Institute of Microbiology).

References

[1] Savage DC. Mucosal microbiota. In: Ogra PL, Mestecky J, Lamm ME, Strober W, Bienenstock J, McGhee J, editors. Mucosal immunology. New York: Academic Press; 1999. p. 19–30.
[2] Berg RD. Bacterial translocation from the gastrointestinal tract. Trends Microbiol 1999;4:430–5.
[3] Henderson B, Wilson M, McNab R, Lax AJ, editors. Cellular microbiology. Chichester: Wiley; 1999.
[4] Boman HG. Innate immunity and normal microflora. Immunol Rev 2000;173:5–16.
[5] Schaedler RW, Dubos R, Costello R. The development of the bacterial flora in the gastrointestinal tract of mice. J Exp Med 1965;122:77–83.
[6] Hooper LV, Gordon JI. Commensal host–bacteria relationships in the gut. Science 2001;292:1115–8.
[7] Salzman NH, de Jong H, Paterson Y, Harmsen HJ, Welling GW, Bos NA. Analysis of 16S libraries of mouse gastrointestinal microflora reveals a large new group of mouse intestinal bacteria. Microbiology 2002;148:3651–60.
[8] Harmsen HJ, Raangs GC, He T, Degener JE, Welling GW. Extensive set of 16S rRNA-based probes for detection of bacteria in human feces. Appl Environ Microbiol 2002;6:2982–90.
[9] Wilson M, McNab R, Henderson B, editors. Bacterial disease mechanisms. Cambridge: Cambridge University Press; 2002.
[10] Rappuoli R. Pushing the limits of cellular microbiology: microarrays to study bacteria–host cell intimate contacts. PNAS 2000;97:13467–9.
[11] Ogra PL, Mestecky J, Lamm ME, Strober W, Bienenstock J, McGhee J, editors. Mucosal immunology. New York: Academic Press; 1999.
[12] Mestecky J, Russel MW, Jackson S, Michalek SM, Tlaskalová-Hogenová H, Šterzl J, editors. Advances in mucosal immunology. New York/London: Plenum Press; 1995.
[13] Fujihashi K, Hirotomo K, van Ginkel FW, Koga T, Boyaka PN, Jackson RJ, et al. A revisit of mucosal IgA immunity and oral tolerance. Acta Odontol Scand 2001;59:301–8.
[14] Tlaskalová-Hogenová H, Tuèková L, Lodinová-Zádníková R, Štìpánková R, Cukrowska B, Funda DP, et al. Mucosal immunity: its role in defense and allergy. Int Arch Allergy Immunol 2002;128:77–89.
[15] Tlaskalová-Hogenová H, Štìpánková R, Tuèková L, Farre MA, Funda DP, Verdu EF, et al. Autoimmunity, immunodeficiency and mucosal infections: chronic intestinal inflammation as a sensitive indicator of immunoregulatory defects in response to normal luminal microflora. Folia Microbiol 1998;43:545–50.
[16] Fasano A. Intestinal zonulin: open sesame! Gut 2001;49:159–62.
[17] Rossi M, Maurano F, Luongo D, Fasano A, Uzzau S, Auricchio S, et al. Zonula occludens toxin (Zot) interferes with the induction of nasal tolerance to gliadin. Immunol Lett 2002;81:217–21.
[18] Walker WA. Development of the intestinal mucosal barrier. J Pediatr Gastroenterol Nutr 2002;34(Suppl 1):S33–9.
[19] Ulevitch RJ. Endotoxin opens the Tollgates to innate immunity. Nat Med 1999;5:144–5.
[20] Medzhitov R, Janeway Jr Ch. Innate immune recognition: mechanisms and pathways. Immunol Rev 2000;173:89–97.
[21] Akira S, Hemmi H. Recognition of pathogen-associated molecular patterns by TLR family. Immunol Lett 2003;85:85–98.
[22] Strober W, Fuss IJ, Blumberg RS. The immunology of mucosal models of inflammation. Ann Rev Immunol 2002;20:495–549.
[23] Bland PW. Mucosal T cell–epithelial cell interactions. Chem Immunol 1998;71:40–63.

[24] Funda DP, Tuèková L, Farre MA, Iwase T, Moro I, Tlaskalová-Hogenová H. CD14 is expressed and released as soluble CD14 by human intestinal epithelial cells in vitro: lipopolysaccharide activation of epithelial cells revisited. Infect Immun 2001;69:3772–81.
[25] Bry L, Falk PG, Midtvedt T, Gordon I. A mode of host–microbial interactions in an open mammalian ecosystem. Science 1996;273:1380–3.
[26] Hooper LV, Wong MH, Thelin V, Hansson L, Falk PG, Gordon JI. Molecular analysis of commensal host–microbial relationships in the intestine. Science 2001;29:881–4.
[27] Bevins CL, Martin-Poter E, Ganz T. Defensins and innate host defence of the gastrointestinal tract. Gut 1999;45:911–5.
[28] Hayday A, Theodoridis E, Ramsburg E, Shires J. Intraepithelial lymphocytes: exploring the third way in immunology. Nat Immunol 2001;2:997.
[29] Powrie F. T cells in inflammatory bowel disease: protective and pathogenic roles. Immunity 1995;3:171–4.
[30] Singh B, Read S, Asseman Ch, Malmstrom V, Mottet Ch, Stephens LA, et al. Control of intestinal inflammation by regulatory T cells. Immunol Rev 2001;182:190–200.
[31] Bos NA, Cebra JJ, Kroese FGM. B-1 cells and the intestinal microflora. Curr Top Microbiol Immunol 2000;252:211–8.
[32] Macpherson AJ, Gatto D, Sainsbury E, Harriman GR, Herngartner R, Zinkernagel RM. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science 2000;288:2222–6.
[33] Fagarasan S, Honjo T. Intestinal IgA synthesis: regulation of front-line body defences. Nat Rev Immunol 2003;3:63–72.
[34] Mestecky J, Russel MW, Elson CO. Gut 1999;44:2–5.
[35] Phalipon A, Cardona A, Kraehenbuhl JP, Edeln L, Sansonetti PJ, Corthésy B. Secretory component: a new role in secretory IgA-mediated immune exclusion in vivo. Immunity 2002;17:107–15.
[36] Denzel A, Hoøejší V, Hayday A. The 5th EFIS Tatra Immunology Conference on 'Molecular determinants of T cell immunity' held in the High Tatra Mountains, Slovakia, September 7–11, 2002. Immunol Lett 2003;86:1–6.
[37] Gustafsson JA. Germfree rearing of rats. Acta Pathol Microb Scand 1948;22(Suppl 1):1–132.
[38] Cebra J. Influences of microbiota on intestinal immune system development. Am J Clin Nutr 1999;69:1046S–51S.
[39] Umesaki Y, Setoyama H, Matsumoto S, Imaoka A, Itoh K. Differential roles of segmented filamentous bacteria and clostridia in development of the intestinal immune system. Infect Immun 1999;67:3504–11.
[40] Pleasants JR, Johnson MH, Wostmann BS. Adequacy of chemically defined, water-soluble diet for germfree BALB/c mice through successive generations and litters. J Nutr 1986;116:1949–64.
[41] Šterzl J, Mandel J, Štípánková R. The use of gnotobiological models for the studies of immune mechanisms. Nahrung 1987;31:599–608.
[42] Cebra J, Jiang HQ, Šterzl J, Tlaskalová-Hogenová H. The role of mucosal microbiota in the development and maintenance of the mucosal immune system. In: Ogra PL, Mestecky J, Lamm ME, Strober W, Bienenstock J, McGhee J, editors. Mucosal immunology. New York: Academic Press; 1999. p. 267–80.
[43] Tlaskalová-Hogenová H, Šterzl J, Štìpánková R, Dlabaè V, Vìtvièka V, Rossmann P, et al. Development of immunological capacity under germfree and conventional conditions. Ann N Y Acad Sci 1983;409:96–113.
[44] Vìtvièka V, Tlaskalová-Hogenová H, Štìpánková R. Effects of microflora antigens on lymphocyte migration patterns in germfree and conventional rats. Effects of microflora antigens on lymphocyte migration patterns in germfree and conventional rats. Folia Biol (Prague) 1983;29:412–8.
[45] Tlaskalová-Hogenová H. Gnotobiology as a tool—an introduction. In: Lefkovits I, editor. Immunology methods manual. London: Academic Press; 1997. p. 1524–9.
[46] Štìpánková R, Šinkora J, Hudcovic T, Kozáková H, Tlaskalová-Hogenová H. Differences in development of lymphocyte subpopulations from gut-associated lymphatic tissue (GALT) of germfree and of natural and immune antibodies in young piglets reared without colostrum. Folia Microbiol 1998;43:531–4.
[47] Tlaskalová H, Kamarýtová V, Mandel L, Prokešová L, Kruml J, Lanc A, et al. The immune response of germ-free piglets after peroral monocontamination with living *Escherichia coli* strain 086. I. The fate of antigen, dynamics and site of antibody formation, nature of antibodies and formation of heterohaemagglutinins. Folia Biol 1970;16:177–87.
[48] Tlaskalová-Hogenová H, Štìpánková R. Development of antibody formation in germ-free and conventionally reared rabbits: the role of intestinal lymphoid tissue in antibody formation. Folia Biol 1980;26:81–93.
[49] Butler JE, Sun J, Weber P, Navarro P, Francis D. Antibody repertoire development in fetal and newborn piglets. III. Colonization of the gastrointestinal tract selectively diversifies the preimmune repertoire in mucosal lymphoid tissues. Immunology 2000;100:119–30.
[50] Bos NA, Jiang HQ, Cebra JJ. T cell control of the gut IgA response against commensal bacteria. Gut 2001;48:762–4.
[51] Tlaskalová-Hogenová H, Èerná J, Mandel L. Peroral immunization of germfree piglets: appearance of antibody-forming cells and antibodies of different isotypes. Scand J Immunol 1981;13:467–72.
[52] Cukrowska B, Kozáková H, Øeháková Z, Šinkora J, Tlaskalová-Hogenová H. Specific antibody and immunoglobulin responses after intestinal colonization of germ-free piglets with non-pathogenic *Escherichia coli* 086. Immunobiology 2001;204:425–33.
[53] Cukrowska B, Lodinová-Zádníková R, Enders C, Sonnenborn U, Schulze J, Tlaskalová-Hogenová H. Specific proliferative and antibody responses of premature infants to intestinal colonization with nonpathogenic probiotic *E. coli* strain Nissle 1917. Scand J Immunol 2002;55:204–9.
[54] Hooper LV, Stappenbeck TS, Hong CV, Gordon JI. Angiogenins: a new class of microbicidal proteins involved in innate immunity. Nat Immunol 2003;4:269–73.
[55] Saemann M, Bohmig G, Zlabinger GJ. Short-chain fatty acids: bacterial mediators of a balanced host–microbial relationship in the human gut. Wien Klin Wochenschr 2002;114:289–300.
[56] Kozáková H, Štìpánková R, Øeháková Z, Kolínská Z. Differences in enterocyte brush border enzyme activities in ageing rats reared in germ-free and conventional conditions. Physiol Res 1998;47:253–8.
[57] Rietschel ET, Kirikae TT, Schade FU, Mamat U, Schmidt G, Loppnow W, et al. Bacterial endotoxin: molecular relationships of structure to activity and function. FASEB J 1994;8:217–25.
[58] Freudenberg MA, Salomao R, Sing A, Mitov I, Galanos C. Reconciling the concepts of endotoxin sensitization and tolerance. Prog Clin Biol Res 1998;397:261–8.
[59] Dziarski R. Recognition of bacterial peptidoglycan by the innate immune system. Cell Mol Life Sci 2003;60:1793–804.
[60] Wagner H. Toll meets bacterial CpG–DNA. Immunity 2001;14:499–502.
[61] Hacker G, Redecke V, Hacker H. Activation of the immune system by bacterial CpG–DNA. Immunology 2002;105:245–51.
[62] van Eden W. Heat-shock proteins as immunogenic bacterial antigens with the potential to induce and regulate autoimmune arthritis. Immunol Rev 1991;121:5–28.
[63] Waksman BH. Immune regulation in adjuvant disease and other arthritis models: relevance to pathogenesis of chronic arthritis. Scand J Immunol 2002;56:12–34.
[64] Kotzin BC, Leung DM, Kappler J, Marrack P. Superantigens and their potential role in human disease. Adv Immunol 1993;54:99–166.
[65] Bachert C, Gevaert P, van Cauwenberge P. *Staphylococcus aureus* superantigens and airway disease. Curr Allergy Asthma Rep 2002;2:252–8.
[66] Abreu MT, Vora P, Faure E, Thomas LS, Arnold ET, Arditi M. Decreased expression of Toll-like receptor-4 and MD-2 correlates with

intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide. J Immunol 2001;167:1609–17.
[67] Steidler L, Hans W, Schotte L, Neirynck S, Obermeier F, Falk W, et al. Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. Science 2000;289:1352–5.
[68] Smith PS, Smythies LE, Mosteller-Barnum M, Sibley DA, Russell MW, Merger M, et al. J Immunol 2001;167:2651–6.
[69] Caramalho I, Lopes-Carvalho T, Ostler D, Zelenay S, Haury M, Demengeot J. Regulatory T cells selectively express Toll-like receptors and are activated by lipopolysaccharide. J Exp Med 2003;197:403–11.
[70] Neish AS. The gut microflora and intestinal epithelial cells: a continuing dialogue. Microbes Infect 2002;4:309–17.
[71] Granucci F, Ricciardi-Castagnoli P. Interactions of bacterial pathogens with dendritic cells during invasion of mucosal surfaces. Curr Opin Microbiol 2003;6:72–6.
[72] Verdu EF, Berèík P, Cukrowska B, Farre-Castany MA, Bouzourene H, Sarage E, et al. Oral administration of antigens from intestinal flora anaerobic bacteria reduces the severity of experimental acute colitis in BALB/c mice. Clin Exp Immunol 2000;120:46–50.
[73] Bach JF. Current concepts of autoimmunity. Rev Neurol 2002;158:881–6.
[74] Shoenfeld Y, Sherer Y, Kalden JR. The expanding world of autoimmunity. Trends Immunol 2002;23:278–9.
[75] Rose NR, Mackay IR. The expanding world of autoimmunity. Cell Mol Life Sci 2000;57:542–51.
[76] Toivanen A, Toivanen P. Reactive arthritis. Curr Opin Rheumatol 2000;12:300–5.
[77] Oldstone MB. Molecular mimicry and immune-mediated diseases. FASEB J 1998;12:255–65;
Oldstone MB. Cell 1990;50:819–20.
[78] Wucherpfennig KW. Infectious triggers for inflammatory neurological diseases. Nat Med 2002;8:455–7.
[79] Duchmann R, Schmitt E, Knolle P, Meyer zum Buschenfelde KH, Neurath M. Tolerance towards resident intestinal flora in mice is abrogated in experimental colitis and restored by treatment with interleukin-10 or antibodies to interleukin-12. Eur J Immunol 1996;26:934–8.
[80] Xavier RJ, Podolsky DK. Microbiology. How to get along—friendly microbes in a hostile world. Science 2000;289:1483–4.
[81] Elson CO, Cong Y. Understanding immune–microbial homeostasis in intestine. Immunol Res 2002;26:87–94.
[82] Sellon RK, Tonkonogy S, Shultz M, Dieleman LA, Grenther W, Balish E, et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun 1998;66:5224–31.
[83] Guarner F, Malagelada JR. Gut flora in health and disease. Lancet 2003;8:1831.
[84] Funda DP, Kaas A, Bock T, Tlaskalová-Hogenová H, Buschard K. Gluten-free diet prevents diabetes in NOD mice. Diabetes Metab Res Rev 1999;15:323–7.
[85] Yacyshyn B, Meddings J, Sadowski D, Bowen-Yacyshyn MB. Multiple sclerosis patients have peripheral blood CD45RO+ B cells and increased intestinal permeability. Dig Dis Sci 1996;41:2493–8.
[86] Mercenier A, Pavan A, Pot B. Probiotics as biotherapeutic agents: present knowledge and future prospects. Curr Pharm Des 2003;9:179–91.
[87] Kalliomaki M, Isolauri E. Role of intestinal flora in the development of allergy. Curr Opin Allergy Clin Immunol 2003;3:15–20.
[88] Seegers JF. Lactobacilli as live vaccine delivery vectors: progress and prospects. Trends Biotechnol 2002;12:508–15.
[89] Lodinová-Zádníková R, Slaviková M, Tlaskalová-Hogenová H, Adlerberth I, Hanson LA, Wold A, et al. The antibody responses in breast-fed and non-breast fed infants after artificial colonization of the intestine with *Escherichia coli* 083. Pediatr Res 1991;29:396–9.
[90] Lodinová-Zádníková R, Cukrowska B, Tlaskalová-Hogenová H. Oral administration of probiotic *Escherichia coli* after birth reduces frequency of allergies and repeated infections later in life (after 10 and 20 years). Int Arch Allergy 2003;131:209–11.