
Strategic Targets of Essential Host-Pathogen Interactions

Francesco Blasi Paolo Tarsia Stefano Aliberti

Institute of Respiratory Diseases, University of Milan, IRCCS Ospedale Maggiore Milano, Milano, Italy

Key Words

Antimicrobial peptides · Innate immunity · Surfactant proteins · Toll-like receptors

Abstract

This review summarizes the present concepts regarding the biological processes that mediate intrinsic and innate host defense against microbial invasion of the lung. Innate immunity is the first line of defense of the higher organisms towards invading pathogens. It accomplishes a wide variety of activities including recognition and effector functions. The innate responses use phagocytic cells (macrophages, monocytes, and neutrophils), cells that release inflammatory mediators (basophils, mast cells, and eosinophils), and natural killer cells. The molecular component of innate responses includes complement, acute-phase proteins, and cytokines. Recognition of pathogen-associated molecular patterns is mediated by the pathogen receptors of the innate immune system, among these molecules toll-like receptors have emerged as fundamental components in the innate immune responses to infection, and a link between innate and adaptive immunity. Additional protection comes from polypeptide mediators of the innate host defense, such as the defensins and other antibiotic peptides. In view of the considerable burden in terms of mortality and morbidity that severe infections still pose worldwide, a better understanding of the biological basis of host-pathogen interactions opens stimulating future treatment perspectives.

Copyright © 2005 S. Karger AG, Basel

Introduction

Infections are still a major health problem worldwide, being associated with significant morbidity and mortality. Since the discovery of penicillin, the traditional approach to infections has been based on the development of novel compounds exerting microbicidal activity. The result is an impressive array of antibacterial, antifungal, antiviral and antiparasitic drugs currently at our disposal for clinical use in contrasting infections. However, antimicrobial treatments are hampered by the ever-increasing problem of resistant strains. Alternative strategies are therefore needed based on a better knowledge of microbial pathogenetic mechanisms and a more complete comprehension of host defense systems. Over the last decade, substantial advances have been made into the understanding of host-pathogen interactions in humans.

The epithelial surfaces of the organism exposed to the environment such as skin, respiratory tract, and gut are a challenge for the host defense system given the impressive amount of potential pathogens they may be exposed to. The respiratory epithelial surface in particular poses a formidable threat given the considerable extension in surface area (approximately 150 m²), and the large amounts of microorganisms contained in the roughly 15,000 liters of air inhaled each day. Whereas the gut is connected in series with the environment, allowing sequential activation of defenses such as salivary amylase, gastric acid, and bile, the alveoli are exposed to the envi-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2005 S. Karger AG, Basel
0025-7931/05/0721-0009\$22.00/0

Accessible online at:
www.karger.com/res

Francesco Blasi, MD
Istituto di Fisiologia e Malattie dell'Apparato Respiratorio
Università degli Studi di Milano, Pad. Litta, IRCCS Ospedale Maggiore di Milano
via F. Sforza, 35, IT-20122 Milano (Italy)
Tel. +39 02 55033763, Fax +39 02 50320628, E-Mail francesco.blasi@unimi.it

ronment in parallel. Each alveolar unit must be self-sufficient in the defense against inhaled threats. In addition, whereas for the gut and skin the objective is the maintenance of a normal flora, sterility is the aim in the alveoli. Lastly, given the presence of only two cell layers between the environment and the bloodstream in the alveoli, the risk of dissemination is greater than for any other body boundary.

Innate Immunity

Much attention has been drawn during recent years towards a better understanding of the innate immunity system. Innate immunity is the first line of defense of the higher organisms towards invading pathogens. It accomplishes a wide variety of activities including recognition and effector functions. The former involves the recognition of structures present in microorganisms that are distinct from self. The effector functions include the release of substances exerting direct antimicrobial properties, and production of cytokines and chemokines that recruit inflammatory cells to the site of infection. A further fundamental aspect is the activation and orientation of adaptive immune responses mediated by lymphocyte priming [1]. Innate responses occur within minutes after invasion of the host, and the extent of activation is the same no matter how many times the infectious agent is encountered. Conversely, the adaptive immunity responses are slower in developing and improve on repeated exposure to a given agent. The innate responses use phagocytic cells (macrophages, monocytes, and neutrophils), cells that release inflammatory mediators (basophils, mast cells, and eosinophils), and natural killer cells. The molecular component of innate responses includes complement, acute-phase proteins, and cytokines [2].

Pathogen Recognition Receptors

Recognition of pathogen-associated molecular patterns is mediated by the pathogen receptors of the innate immunity system. From a functional standpoint, these receptors may be divided into three classes: secreted, endocytic and signaling [3]. Among the best characterized in the first group is mannose-binding lectin (MBL), the second group includes macrophage mannose receptors and macrophage scavenger receptors, whereas the third group includes toll-like receptors (TLRs).

Mannose-Binding Lectin

MBL is a complement-activating innate immune defense serum protein that binds to mannose and acetylglucosamine sugar groups present on glycolipids and glycoproteins on microbe surfaces but not on mammalian surface cells [4]. It is a member of a family of calcium-dependent lectins, the collectins (collagenous lectins). MBL is synthesized in the liver and secreted into the serum as a component of the acute-phase response. As other members of the collectin family, the human MBL protein maps to the long arm of chromosome 10. MBL assembles into 18-subunit oligomers aligned in a 'flower bouquet' pattern. It binds to carbohydrates on Gram-positive and Gram-negative bacteria, yeast, viruses and parasites. MBL is associated with two serine proteases, mannan-binding lectin-associated proteases 1 and 2 (MASP1 and MASP2). Following binding with microorganisms, MASP2 cleaves and activates complement components C4 and C2, whereas MASP1 may cleave C3 directly [5]. The result is an activation of the complement cascade leading to the formation of the membrane-attack complex (fig. 1). The MBL pathway of complement activation does not require the presence of host antibody responses. It is triggered directly by microbial recognition and is therefore independent of adaptive immune responses.

Three polymorphisms have been identified within the gene (*MBL-2*) encoding the MBL polypeptide [6]. Allelic variants prevent the assembly of MBL oligomer subunits, thereby reducing the amount of functionally active protein and shortening its half-life. The homozygous-deficient state is present in 3–4% of individuals, and is associated with profoundly reduced levels of MBL, whereas the heterozygote state (roughly 30% of the general population) presents intermediate levels [7]. MBL variant alleles have been associated with an increased risk of recurrent respiratory infections, particularly during early childhood [8]. The mannose-binding pathway may be particularly important during the interval between the loss of maternal antibody coverage and the development of a mature immunological system. In a recent study conducted on cystic fibrosis (CF) patients, Davies et al. [9] showed that the presence of two structural MBL mutations was associated with reduced lung function and oxygen saturation levels, more frequent hospital admissions, and raised systemic inflammatory markers compared to wild-type alleles. A further recent case-controlled study associated mutant genotypes of the MBL gene with increased susceptibility to invasive pneumococcal disease in adult patients [10].

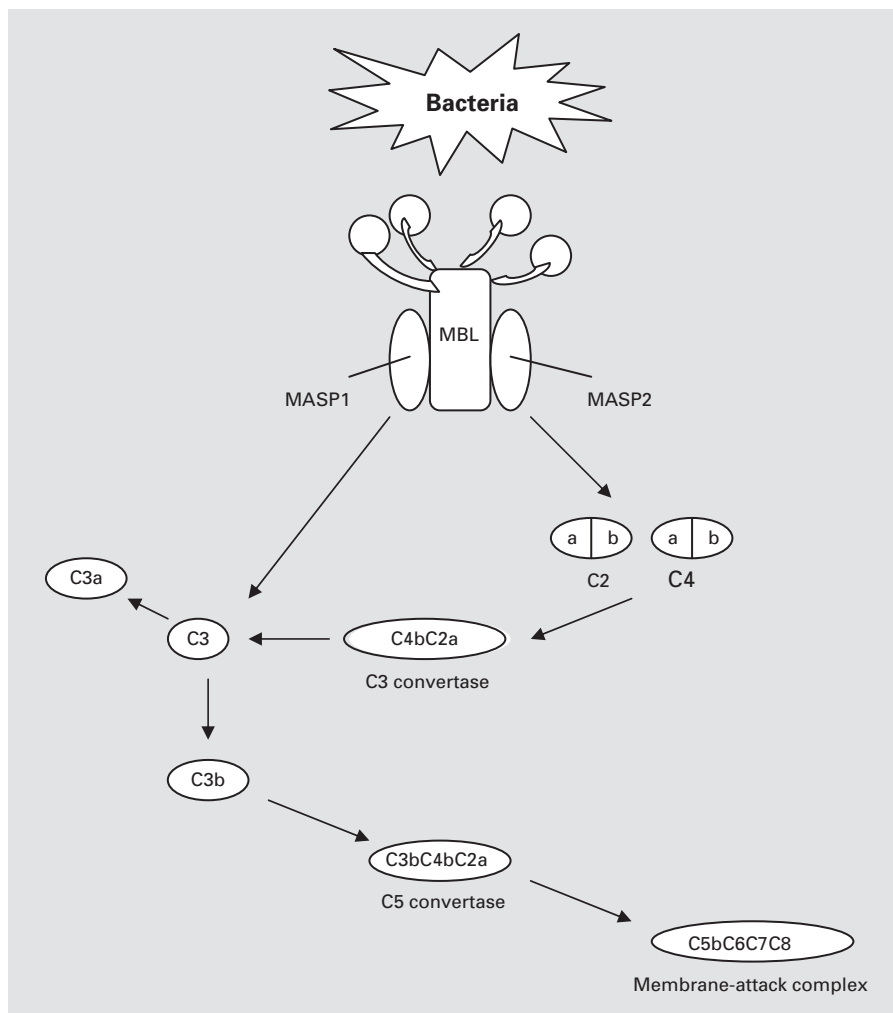


Fig. 1. The mannose-binding lectin (MBL) pathway of complement activation. MBL is a serum protein that binds to mannose sugar groups present on microbial surfaces but not on mammalian cells. It is associated with two serine proteases (MASP1 and MASP2). Binding with microbes activates MASP2 to cleave C4 and C2 complement components, whereas MASP1 may cleave C3 directly. The end result is activation of the membrane-attack complex resulting in cell death.

However, there is also evidence that low levels of MBL partially protect against mycobacterial infections [11]. Since mycobacteria are intracellular pathogens, bacterial opsonization by MBL may actually favor infection by increasing the uptake of the pathogen within macrophages. In this context, low levels of MBL, by reducing macrophage phagocytosis, may confer resistance to mycobacteria.

Attempts are currently underway to make use of MBL as a target for modifier-gene therapy. Purified serum-derived MBL has been administered to a patient with CF with apparent benefit [12]. Considering the significant advances obtained in recombinant protein technology, it is possible to hypothesize that, in a variety of different clinical settings, supplementary MBL may become a treatment option for patients with deficient protein levels.

Macrophage Mannose and Scavenger Receptors

Endocytic pattern-recognition receptors are present on the surface of phagocytic cells such as macrophages and neutrophils. Activation of these receptors favors pathogen uptake and engulfment into lysosomes. Examples include the macrophage mannose receptor and the macrophage scavenger receptor. The former is a member of the calcium-dependent lectin family. It recognizes carbohydrates with large numbers of mannoses, and mediates pathogen phagocytosis by macrophages [13]. Mannoses are sugars that are not normally exposed on the cell surfaces of vertebrates, and therefore their recognition indicates the presence of a microbial pathogen. The macrophage scavenger receptor binds to bacterial cell walls and plays an important role in clearing bacteria from the circulation [14]. Engulfed microorganisms are exposed to a wide variety of intracellular toxins including superoxide

Table 1. Currently identified human TLR, recognized ligands, and microbial sources

TLR	Ligands	Microbial sources
TLR1	triacyl lipopeptides	bacteria and mycobacteria
TLR2	LPS	Gram-negative bacteria
	lipoteichoic acid	Gram-positive bacteria
	peptidoglycan	Gram-positive bacteria
	lipopeptide	Gram-positive bacteria
	zymosan	yeast
	lipoarabinomannan	mycobacteria
TLR3	double-stranded RNA	viruses
TLR4	LPS	Gram-negative bacteria
	RSV coat protein F	viruses
	HSP-60	<i>Chlamydiae</i> or endogenous
TLR5	flagellin	Gram-positive and -negative flagellated bacteria
TLR6	diacyl lipopeptides	bacteria
TLR7	single-stranded RNA	viruses
TLR8	single-stranded RNA	viruses
TLR9	CPG unmethylated DNA	bacteria

anion, hydroxyl radicals, hypochlorous acid, nitric oxide, antimicrobial cationic proteins and peptides, and lysozyme. In addition to invading pathogens, these receptors also identify molecules expressed on the surface of apoptotic host cells, such as phosphatidylcholine, thus favoring phagocytosis of these elements [15].

Toll-Like Receptors

Since their identification roughly 20 years ago, TLRs have emerged as fundamental components in the innate immune responses to infection, and a link between innate and adaptive immunity. They are capable of distinguishing pathogens from self components, triggering cytokine production, and expressing costimulatory molecules necessary for lymphocyte activation. Central to the signaling cascade triggered by TLRs is the activation of transcription factors such as nuclear factor κ B (NF κ B) and activator protein-1 (AP-1), key regulators of inflammatory and immune responses.

The so-called *Toll* gene was first involved in the pathway of dorsal-ventral polarity in *Drosophila melanogaster* fly embryos [16]. The name derives from one of the author's surprise in observing the hatched embryos and exclaiming 'Toll!' (a German exclamation comparable to cool). It was later recognized that the *Toll* gene was also fundamental in immune responses, as mutations in this gene were associated with decreased survival to bacterial and fungal infections in adult flies [17]. Toll-like protein

homologues to the *Drosophila* original have now been described in insects, plants, reptiles, birds, and mammals, indicating that these receptors represent a fundamental host defense mechanism that has been highly conserved over the last 350 million years of evolution.

Human Toll-Like Receptors

So far, nine well-characterized human TLRs have been identified, in addition to several other only partially mapped receptors (table 1). Toll-like receptors are members of the type I transmembrane proteins. The extracellular domain contains multiple copies of leucine-rich repeats and cysteine-rich regions. The intracellular domain presents structural homology with the intracellular domain of the interleukin-1 receptor (IL-1R), and both are now considered part of the IL-1R/TLR superfamily [18]. The extracellular TLR domains recognize membrane-derived structures in invading microorganisms called pathogen-associated molecular patterns that are not found on mammalian cells. They are typically highly conserved and essential structures that play a fundamental role in microbial survival or pathogenic activity. Examples include lipopolysaccharide (LPS) from Gram-negative bacteria, peptidoglycan, teichoic and lipoteichoic acids from Gram-positive bacteria, lipoglycan from *Mycobacterium tuberculosis*, mannans from yeasts, and viral surface proteins.

TLR1 is expressed ubiquitously and at rather high levels. It recognizes triacyl lipopeptides from bacteria and mycobacteria. In addition, it may form heterodimers with TLR2 and TLR6. TLR2 recognizes various microbial products from Gram-negative bacteria (LPS), Gram-positive bacteria (lipoteichoic acid, peptidoglycans, and lipopeptides), yeast (zymosan), mycobacteria (lipoarabinomannan and mannosylated phosphatidylinositol) [19]. The receptor is expressed in peripheral blood mononuclear cells and lymphoid tissue. TLR3 is expressed in the lung, muscle, brain, intestinal cells, and, most importantly, specific subsets of dendritic cells [20]. It is involved in the response to double-stranded RNA that is produced during viral infections [21].

TLR4 is perhaps the best-characterized and most complex TLR. It is displayed on epithelial surfaces, dendritic cells, and macrophages. The best-known TLR4 ligand is undoubtedly LPS, although additional ligands include lipoteichoic acids, mycobacterial proteins, glycolipids, respiratory syncytial virus protein F, and non-bacterial elements such as heat shock protein 60 and taxol [22–24]. LPS exerts the most potent immunostimulatory activity of the TLR ligands. TLR4 activation by LPS is a complex

event that requires the presence of numerous other elements. LPS is first bound by the lipopolysaccharide binding protein (LBP), a 60-kDa plasma lipid transfer glycoprotein. LBP-bound LPS is carried away from microbial surfaces and shuttled into contact with CD14 [25]. This is a 50-kDa glycoprotein that is either expressed on the surface of myelomonocytic cells or present as a soluble molecule in the bloodstream. Surface CD14 is devoid of an intracellular domain, and LPS processing by this molecule is probably not essential for host responses, although it has a role in their amplification and modulation. In fact, in addition to activating TLR4, it has recently been recognized that both LBP and soluble CD14 can catalyze the movement of LPS to high-density lipoprotein (HDL) particles in which the endotoxin loses its biological activity and is then excreted from the liver [26]. TLR4 works downstream of CD14 and is responsible for activating a response signal to LPS. However, in order to be fully active, TLR4 must be associated with MD-2, a surface molecule with no intracellular domain [27]. Association between TLR4 and MD-2 occurs in the endoplasmic reticulum, in the absence of which processing of TLR4 does not progress further than the Golgi apparatus [28]. MD-2 appears to be involved in the final glycosylation processes of the TLR4 molecule that allow it to reach the cell surface [29]. Once on the cell surface, MD-2 appears to play an active role in receptor signaling following contact with LPS by regulating TLR4 dimerization [30]. MD-2 may possess a specific role in LPS recognition that contributes to the modulation of the proinflammatory response of effector cells [31].

TLR5 appears to be the principal means through which the innate immunity system recognizes flagellated bacteria. Motility is an important virulence factor for bacteria such as *Salmonella* spp., *Pseudomonas aeruginosa*, and *Listeria monocytogenes*. Bacterial flagellin from either Gram-positive or Gram-negative bacteria is the major ligand for TLR5 [32]. TLR6 recognizes bacterial diacyl lipopeptides, whereas TLR7 and TLR8 are involved in recognition of single-stranded RNA viruses.

Microbial DNA contains repetitive 2M-deoxyribo-(cytidine-phosphate-guanosine) (CpG)-rich unmethylated motifs, whereas these sequences are relatively rare in mammalian DNA. Bacterial DNA is known to be a potent immunostimulant for mammalian cells, and TLR9 has been identified as the ligand for CPG repeats on immune effector cells [33].

TLRs may also aggregate in heterodimers (e.g. TLR2-TLR1 or TLR2-TLR6) in distinguishing between different pathogen-associated molecular patterns [34].

Toll-Like Receptor Signal Activation

Engagement of the extracellular domain of TLRs by microbial products activates intracellular signal transduction cascade pathways leading to the liberation of transcription factors (fig. 2). Following ligand activation, TLRs likely form homodimers, thus resulting in a conformational change of the intracellular Toll-IL-1 receptor (TIR) domain that forms an active signaling complex. This complex consists of a myeloid differentiation factor (MyD88) and a TIR-domain-containing adaptor protein (TIRAP or Mal) [35]. MyD88 recruits IL-1-receptor-associated kinases (IRAKs) to the complex [36]. Subsequent activation of IRAKs is controlled by both positive regulators (such as IRAK-4) [37] and negative regulators (such as IRAK-M) [38]. Following autophosphorylation, IRAKs dissociate from the complex and associate with tumor necrosis factor (TNF)-receptor-associated factor 6 (TRAF6). TRAF6 plays a pivotal role in downstream signal transduction and exerts fundamental functions in innate and adaptive immunity, inflammation and tissue homeostasis [39]. The biological effects of TRAF6 are mediated by activation of either the NF κ B kinase (NIK), which modulates the transcriptional activity of NF κ B, or activation of mitogen-activated protein (MAP) kinases, which regulate AP-1 transcription. Recruitment of both pathways involves a group of adapter proteins such as transforming growth factor (TGF)- β -activated kinase 1 (TAK1) and binding proteins (TAB1 and TAB2) [40]. The TRAF6-TAK1-TAB1-TAB2 complex translocates from the cell membrane to the cytosol and activates NIK and MAP kinases. The former is a hetero-trimeric enzyme containing two kinase subunits (IKK α and IKK β) and a regulatory subunit (IKK γ /NEMO).

Following activation by the TRAF6 complex, NIK phosphorylates and degrades I κ B, the inhibitor of NF κ B, leading to translocation of the transcription factor into the nucleus. The activated form of NF κ B is a heterodimer, which usually consists of two proteins, a p65 (also called relA) subunit and a p50 subunit. Other subunits, such as relB and p52, may also be part of activated NF κ B, and it is likely that the different forms of NF κ B may activate different sets of target genes. NF κ B regulates the expression of several genes that encode proinflammatory cytokines (e.g. IL-1 β , TNF- α , IL-6, and granulocyte-macrophage colony-stimulating factor), many chemokines (e.g. IL-8, macrophage inflammatory protein-1 α , macrophage chemotactic protein-1, and eotaxin), in addition to adhesion molecules (e.g. intercellular adhesion molecule-1, vascular-cell adhesion molecule-1, and E-selectin), and inflammatory enzymes (e.g. inducible nitric oxide syn-

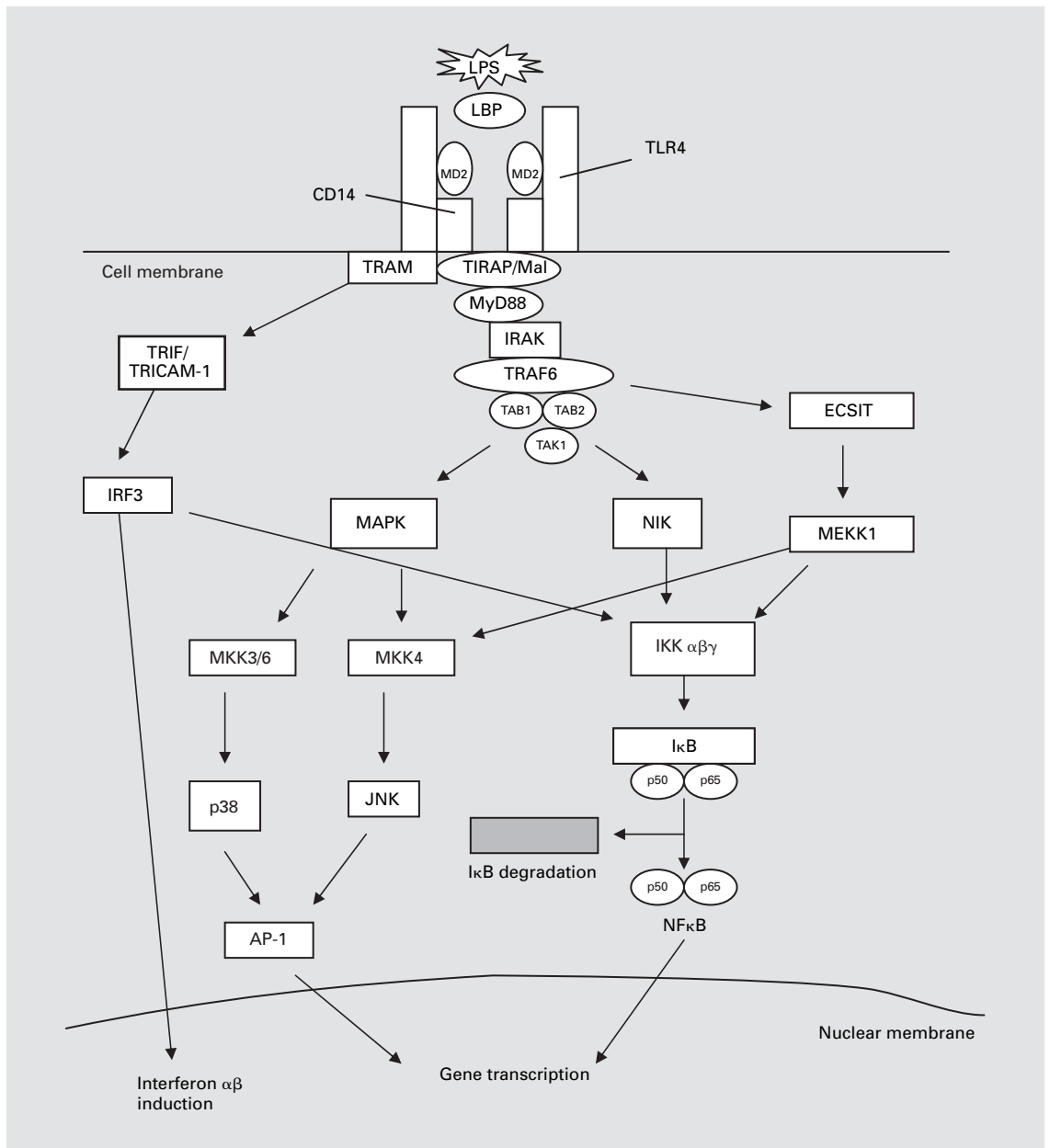


Fig. 2. Signaling pathway following activation of TLR4 by LPS.

these, inducible cyclooxygenase-2, 5-lipoxygenase, and cytosolic phospholipase A₂). It has also been shown that NF κ B plays a role in regulating antimicrobial peptides such as β -defensins [41]. Lastly, NF κ B transactivation mediated by TLR4 induces expression of CB80 and CD86, costimulatory molecules that must be expressed on the surface of dendritic cells in order to activate antigen-specific responses in naïve T cells. This provides a link between innate and adaptive immunity.

Alternatively, I κ B degradation, and consequent NF κ B liberation, may be brought about by a MAP kinase/ERK kinase 1 (MEKK1) [42]. Processing MEKK1 into its active form is aided by binding of TRAF6 with another protein, termed evolutionarily conserved signaling intermediate in Toll pathway (ECSIT) [43]. MEKK1 is also capable of activating the MAP kinase pathway, involving MKK4, which in turn recruits c-Jun NH₂-terminal kinase (JNK) leading to AP-1 liberation [44]. Alternative me-

diators in the MAP kinase pathway activated by TRAF6 are MKK3/MKK6, which activate the p38 cascade resulting in AP-1 liberation [44].

TLR4 stimulation is able to activate an additional MyD88-independent signaling pathway. This involves a TIR-domain-containing adapter-inducing interferon (IFN)- β (TRIF or TICAM-1) and a TRIF-related adapter molecule (TRAM) [45]. These adapters, like MyD88, contain a TIR domain, but their overall structure is unrelated to MyD88. This independent pathway activates IFN regulatory factor 3 (IRF3), leading to the induction of IFN- α and - β , and initiates NF κ B transcription factors.

Toll-Like Receptor Activation Escape Mechanisms

Activation of TLRs following contact with pathogen-associated molecular patterns is undoubtedly an important step in marshaling host inflammatory defenses against infection. NF κ B is rapidly activated following microbial invasion, and this activation is associated with resistance to infection. Nonetheless, persistent activation of transcription factors may result in excessive production of proinflammatory cytokines, leading to tissue damage, hypotension, organ failure, and ultimately to death in such extreme conditions as septic shock [46]. The TLR system is normally finely tuned, so that TLRs are also involved in activating the release of anti-inflammatory cytokines such as IL-10, and IL-13 [47] and recruiting downregulating T-regulatory cells [48]. In particular, TLR2 activation seems to provide, among others, signals involved in shutting off inflammation once the invading microorganisms have been eliminated. This includes induction of a TH-2 type cytokine response, inhibition of IFN- γ signals, and induction of apoptosis [49]. Certain pathogens, such as *Candida albicans*, *Yersinia enterocolitica*, and mycobacteria appear to have developed a strategy of premature TLR2-mediated anti-inflammatory activation in order to evade host defense mechanisms [50].

Other pathogens have developed strategies to block or avoid their recognition by TLRs. This may be achieved through phospholipid constituents that block the function of LBP or CD14 [51]. Alternatively, certain viruses produce proteins that associate with IRAK or TRAF6 proteins, blocking downstream liberation of NF κ B [52]. *Helicobacter pylori* may modify its flagellin component, evading recognition by TLR5 [53]. Lastly, viruses such as HIV-1 and CMV may take advantage of TLR-mediated NF κ B activation to favor transcription of their own genes, thus enhancing infectivity [54].

Toll-Like Receptors as Potential Therapeutic Targets

Given their central role in activating and modulating host responses to infection, and acting as a bridge between innate and adaptive immunity, TLRs are being currently studied as potential therapeutic targets. Soluble forms of TLRs may be made to bind and neutralize natural ligands before they activate potent proinflammatory responses in the host. Examples may be soluble TLR4 in Gram-negative sepsis and soluble TLR2 in Gram-positive toxic shock [55]. Alternatively, antibodies or molecules similar to natural ligands could be employed in order to bind with extracellular domains of TLRs, though failing to activate intracellular signaling [56]. Lastly, interference with the intracellular TLR domain, adapter molecules such as MyD88, or key kinases such as IRAK and TRAF6 may be studied as future therapeutic targets in conditions such as sepsis, where treatment may be aimed at blocking proinflammatory cascades initiated by TLR, while retaining TLR-related protective responses [57].

Soluble Factors in Innate Immunity

C-Reactive Protein

Acute-phase proteins enhance resistance to infection and promote the repair of damaged tissue [58]. C-reactive protein (CRP) was the first acute-phase protein to be described. It belongs to the pentraxin family of calcium-dependent ligand-binding plasma proteins and is produced by hepatocytes, under the effects of IL-6. When the levels of this cytokine are increased as during infection, inflammation, tissue damage, and malignancy, CRP synthesis is upregulated in the liver causing a rapid surge in plasma levels. CRP is composed of five identical non-glycosylated polypeptide units non-covalently bound in an annular configuration with cyclic pentameric symmetry [59]. It binds to a series of ligands, including glycan and phospholipid elements in somatic and capsular components of bacteria, fungi, and parasites. When bound to ligands, CRP is recognized by the C1q and potently activates the classical component pathway, thus favoring pathogen lysis and phagocytosis. CRP may thus contribute to host defenses against infection, and function as a proinflammatory mediator. However, in certain clinical circumstances it may contribute to the pathogenesis of disease by exacerbating tissue damage leading to more severe inflammatory derangement. It has therefore been postulated that CRP may be a target in downregulating host responses to infection. A series of novel drugs are under development that spe-

cifically block CRP binding and its proinflammatory effects [60].

Respiratory Tract Epithelium in Innate Immunity

Epithelial surfaces provide a physical barrier separating the host from the environment. The airway epithelial surface is covered by the airway surface liquid. It consists of two layers, a mucus layer and a periciliary liquid layer (PCL). The mucus layer consists of high-molecular-weight, heavily glycosylated macromolecules that behave as a tangled network of polymers. Mucin macromolecules present extraordinary diversity of their carbohydrate side chains facilitating non-specific binding to pathogens that land on airway epithelia and can thus clear them from the lung [61]. The PCL provides a low-viscosity solution in which cilia can beat rapidly. In addition to its propelling action on the overlying mucus layer, ciliary beat also imparts vertical movements within the mucus layer, thus allowing rapid mixture of pathogens deposited on the mucus surface. Cough represents an additional mechanism to clear mucus from the lung. However, clearance of bacteria from the peripheral airways by mucus transport at the rate of 60 $\mu\text{m/s}$ may require up to 6 h. Considering that bacterial numbers may double every 20 min, a large bacterial burden may accumulate in the airways during the 6-hour clearance period [61]. The epithelium is capable of secreting antimicrobial factors capable of suppressing bacterial growth for the short period required to clear bacteria from the airways by mucus transport (see below).

Alterations in airway surface liquid features are important in host-pathogen interactions in different disease conditions. For example, derangement of epithelial function in CF patients associated with CF transmembrane conductance regulator (CFTR) mutations results in PCL volume depletion. Normal epithelia respond to PCL volume depletion by slowing Na^+ absorption and inducing Cl^- secretion [62]. In CF airway epithelia there is an accelerated basal rate of Na^+ absorption due to the absence of the inhibitory effect of CFTR. In addition, epithelia also lack the capacity to add liquid back to the airway surface due to the absence of CFTR functioning as a channel. Resulting PCL depletion allows gluing of mucus layer mucins to mucins of the cell surface (the 'Velcro effect') thus abolishing cough efficacy. Furthermore, loss of PCL volume removes the liquid in which cilia extend and beat, inhibiting mucociliary clearance. Thick airway secretions and hypoxic zones caused by high O_2 consumption due

to increased Na^+ transport facilitate *P. aeruginosa* colonization with alginate production and biofilm formation [63]. Therapies directed at slowing the abnormal volume absorption (Na^+ channel blockers) and initiating Cl^- channel activity provide routes for the treatment of the primary defect in the CF airway epithelium [64]. Other attempts to 'rescue' the mutant protein with drug interventions are underway. Oral administration of 4-phenylbutyrate produces modest correction of the bioelectric defect in CF patients [65]. Ingestion of curcumin (a component of tumeric) was recently discovered to correct the bioelectric defect of CFTR in mice [66]. Biofilm fouling mechanisms (e.g. naturally occurring furanones) capable of inhibiting bacterial quorum-sensing systems have been isolated from the marine macro alga *Delisea pulchra*. Synthetic furanone compounds have been developed and have been shown to inhibit bacterial quorum-sensing in *P. aeruginosa* and exhibited favorable therapeutic effects on *P. aeruginosa* lung infection in an animal model [67].

Antimicrobial Activity of Airway Secretions

The airway surface fluid contains a number of substances that exert antimicrobial activity. These include enzymes, protease inhibitors, binding proteins, and a number of more recently identified peptides known as antimicrobial peptides (AMPs). AMPs are effector molecules of the innate immunity with direct antimicrobial and mediator function. They provide an initial host defense mechanism that protects mucosal and dry epithelial surfaces of all multicellular organisms [68]. AMPs are an evolutionary ancient weapon, and have played an important role in the successful evolution of multicellular organisms. The main classes of AMPs include defensins, cathelicidins, and histatins. They have a broad spectrum of activity towards bacteria, fungi and viruses. The minimal inhibitory concentrations of these peptides are in the range of 0.1–100 $\mu\text{g/ml}$ [69]. Most AMPs are cationic molecules with spatially separated hydrophobic and charged regions that show electrostatic attraction to anionic microbial surfaces. In many cases, AMP are able to kill bacteria by depolarizing and permeabilizing anionic lipid bilayers, resulting in the release of cellular contents and the destruction of the electrical membrane potential. In Gram-negative bacteria, peptides first bind with negatively charged moieties of the outer membrane, producing structural cracks and pore formation. Disruption of barrier function and integrity of the outer membrane allows passage of AMPs and other molecules to internal targets on the cytoplasmic membrane. Gram-positive

Table 2. Main activities and sources of innate antimicrobial peptides

Antimicrobial peptide	Activity	Source
Lysozyme	enzymatic	epithelium, neutrophils, macrophages
Lactoferrin	iron-binding (bacteriostatic) antimicrobial (bactericidal)	epithelium, neutrophils
SLPI	antimicrobial neutrophil elastase inhibitor	epithelium
Elafin	antimicrobial neutrophil elastase inhibitor	epithelium
BPI	LPS binding (anti-inflammatory)	epithelium, neutrophils
PLUNC	LPS binding (anti-inflammatory)	upper and lower airway epithelia
Defensins α-Defensins (HNP1–4) α-Defensins (HNP5, HNP6) β-Defensins (HBD1–4)	membrane depolarization (Gram-positive and -negative bacteria, yeast, fungi, enveloped viruses)	neutrophils small intestine and urogenital epithelium epithelium, macrophages, dendritic cells
Cathelicidins (LL-37)	LPS binding	epithelium, neutrophils
Histatins (1,3, and 5)	antifungal	salivary glands
Hepcidin	in vitro activity towards Gram-negative bacteria and fungi	liver
Indolicidin	modified analogues, potent antimicrobials	bovine neutrophils
Neuropeptides Vasostatin-I Enkelytin Ubifungin	antibacterial, antifungal	neutrophils, chromaffin cells

SLPI = Secretory leukoproteinase inhibitor; BPI = bactericidal permeability-increasing protein; PLUNC = palate, lung, and nasal epithelium clones; LPS = lipopolysaccharide; HNP = human neutrophil peptides; HBD = human β-defensin.

bacteria lack an outer membrane, but AMPs bind to external components such as lipoteichoic acids. The cholesterol and neutral phospholipid-rich cell membranes of the host are relatively spared. The antimicrobial activity of polypeptide AMPs is rather precarious and may be inhibited simply by altering ion concentrations in respiratory secretions.

Both epithelial cells and polymorphonuclear leukocytes produce an array of antimicrobial substances. In some cases (e.g. lysozyme and lactoferrin), the same genes are highly expressed in both cell types; in other cases (e.g. defensins), the two cell types express different members of the same gene family [70]. An important aspect of AMP activity is that they form a part of a cocktail of antimicrobial substances that work synergistically to combat infection. In fact, it is well known that taken singularly, many AMPs are inhibited by increasing concentrations of NaCl.

However, synergy between defensin and cathelicidin compounds is capable of overcoming the inhibitory effects of salt [71]. Table 2 contains a list of antimicrobial peptides, indicating prevalent activity and cellular source.

Lysozyme

Lysozyme is an enzyme produced by neutrophil granules, epithelial cells, and monocytes/macrophages. Its enzymatic activity breaks up N-acetyl residue bonds in peptidoglycan layers. Lysozyme therefore exerts antimicrobial effects against a wide range of Gram-positive microorganisms [72]. However, the presence of cofactors, e.g. lactoferrin, disrupts the outer membrane of Gram-negative bacteria, allowing lysozyme access to the underlying peptidoglycan layer. Together with lactoferrin, lysozyme is the most abundant antimicrobial protein of airway secretions (0.1–1 mg/ml).

Lactoferrin

This iron-binding protein is closely linked to transferrin, and is produced in human neutrophil granules and respiratory secretions. It inhibits microbial growth by sequestering iron essential for microbial respiration [73]. Besides this bacteriostatic action, lactoferrin can show a direct bactericidal activity due to its binding to the lipid A part of bacterial LPS, with an associated increase in membrane permeability. This action is due to lactoferricin, a peptide obtained from lactoferrin by enzymatic cleavage, which is active against bacteria, fungi, protozoa and viruses. Additional antibacterial activities of lactoferrin include specific effects on biofilm development, bacterial adhesion and colonization, intracellular invasion, apoptosis of infected cells, and neutrophil bactericidal activity [74]. The future perspectives of this peptide appear to be linked to its potential prophylactic and therapeutic use in a considerable spectrum of medical conditions, taking advantage of the availability of a recombinant human lactoferrin.

Secretory Leukoprotease Inhibitor

Secretory leukoprotease inhibitor is about tenfold less abundant in respiratory secretions compared to lysozyme and lactoferrin. It is a non-glycosylated protein consisting of two domains. The N-terminal domain displays modest antimicrobial activity towards Gram-positive and Gram-negative bacteria. The C-terminal domain acts as an effective inhibitor of neutrophil elastase and may also be involved in intracellular regulation of response to LPS [75]. Elafin (elastase-specific inhibitor) is a related inhibitor of neutrophil proteinase activity, and is also directly antimicrobial [76].

Bactericidal Permeability-Increasing Protein

Bactericidal permeability-increasing protein (BPI) is expressed on the cell membranes of human epithelial cells and is an abundant constituent of neutrophils [77]. BPI is bactericidal to many Gram-negative bacteria through avid binding to LPS, thus killing bacteria that become tightly adherent to epithelial cells. The antimicrobial function of BPI is mediated by its N-terminal domain, which is similar in structure and function to other innate cationic AMPs [78].

In addition, BPI is structurally related to the LBP present in blood plasma that helps to activate the inflammatory response to Gram-negative bacteria. LBP and BPI may be considered to have antagonistic functions. Whereas LPS binding to LBP enhances cellular responses to LPS, BPI binding to LPS dampens cellular responses. BPI

may be considered an anti-inflammatory protein that, through competition with LBP for LPS, inhibits the signals that would provoke a potentially damaging inflammatory response in the airways [77].

Recently, a novel family of protein products (palate, lung, and nasal epithelium clones – PLUNC) showing analogy to LBP and BPI have been identified. The *PLUNC* genes in humans are located in a compact cluster on chromosome 20 [79]. Similarly to BPI, PLUNC proteins may exert anti-inflammatory activity by binding to LPS and blunting early activation of the inflammatory cascade [80].

Defensins

Human defensins are 3.5- to 4.5-kDa non-glycosylated peptides containing cysteine residues and disulfide bridges that allow the distinction into two classes: α -defensins and β -defensins [81]. The genes encoding α -defensins and β -defensins are present on chromosome 8p23 [82]. α -Defensins were first discovered in the azurophilic granules of neutrophils and named human neutrophil peptides 1–4 (HNP-1 to HNP-4). Neutrophils that migrate to the airways during infection or inflammation produce abundant quantities of HNP1–4 [83]. Additional human defensins 5 and 6 were later found in Paneth's cells of the small intestine and epithelial cells of the urogenital tract.

In humans, β -defensins are produced by all epithelial tissues. Four human β -defensins have been identified to date. Human β -defensin-1 (HBD1) is constitutively produced in epithelial cells of the urinary and respiratory tract [84]. The remaining defensins (HBD2 to HBD4) are induced by transcription factor κ B (NF κ B) following LPS activation of the CD14/TLR4 complex [85]. Additionally, the macrophage IL-1 α and IL-1 β response to microbial encounter activates epithelial receptors to upregulate defensin synthesis [86]. Mucoid *P. aeruginosa* strains appear to activate HBD2 production in normal but not in CF epithelia [87].

Defensins exert antimicrobial activity against a wide variety of pathogens (Gram-positive and Gram-negative bacteria, yeast, fungi, and enveloped viruses). They act preferentially on microbes by permeabilizing cell membranes rich in anionic phospholipids. Inducible HBD2 is much more potent than constitutive HBD1 as an antimicrobial agent. The latter may play a greater role in binding and neutralizing LPS and lipoteichoic acids, thus helping prevent inordinate immune responses to low levels of organisms or resident commensal bacteria.

Cathelicidins

Cathelicidins are a large and ancient family of mammalian antimicrobial peptides with a conserved N-terminal structure and heterogeneous C-terminal domain [88]. The only human cathelicidin identified to date is LL-37 (or hCAP-18). The gene encoding LL-37 is localized on chromosome 3. LL-37 is present in neutrophil granules but may also be secreted into epithelial fluid by the same cell types as β -defensins [89]. Cathelicidins have the ability to bind to LPS and inactivate the biological functions of this endotoxin. Similarly to β -defensins, LL-37 levels increase in the airways following infection or inflammation due to increase in both neutrophil and epithelial production [90]. The gene encoding LL-37 contains several potential binding sites for transcription factors that may be involved in regulating gene expression.

In vivo experimental models have been used to test the importance of AMP in the defense of the respiratory tract. Overexpression of the human cathelicidin LL-37 in a CF mouse model resulted in increased killing of *P. aeruginosa*, reduced ability of this bacterium to colonize the airways, reduced inflammation and reduced susceptibility to shock [91].

Histatins

Histatins are a family of histidine-rich peptides secreted by salivary glands into human saliva [92]. At least 12 forms of salivary histatin have been detected, but the family members of primary importance in host defense are histatins 1, 3, and 5. The genes that encode histatins have been mapped to chromosome 4q13. Their antimicrobial activity includes strong antifungal effects, particularly towards *Candida* spp. More recently, histatins have also been shown to inhibit some Gram-positive bacteria, and suppress cytokine induction [93].

Additional Biological Activities of Antimicrobial Peptides

In addition to acting as endogenous antibiotics by exerting direct antimicrobial activity, AMPs possess broad biological activities indicating they are effector molecules providing communication between innate and adaptive immune responses [94]. These biological activities include regulation of inflammation (e.g. cytokine release and chemotaxis), wound repair, protease-antiprotease balance, redox homeostasis, and adaptive immunity activation. Defensins and cathelicidins may exert proinflammatory activities by inducing cytokine and chemokine secretion from a variety of immune cells and epithelial cells [95]. AMP activation may therefore favor the

influx of neutrophils, macrophages, and T cells into the airways. An important property of AMPs is their ability to bind avidly to many proinflammatory molecules released from microorganisms such as LPS, lipoteichoic acid and DNA. By binding to these molecules, AMPs inhibit responses of host cells and dampen undesirable inflammatory responses.

Defensins may also play a role in regulating protease-antiprotease balance by regulating secretory leukoprotease inhibitor and elafin secretion from epithelial cells [96]. Furthermore, redox homeostasis may be influenced by defensin interference with intracellular glutathione levels [97].

Neutrophil α -defensins may contribute to epithelial repair in the respiratory tract by enhancing lung epithelial cell proliferation [98] and exerting mitogenic effects on fibroblasts [99]. Cathelicidins also appear to be involved in promoting epithelial growth and angiogenesis [100].

Defensins have been shown to display chemoattractant effects on immature dendritic cells and memory T cells, thus rendering them an important potential link between innate and acquired immune response to infection [101].

Resistance to Antimicrobial Peptides

Notwithstanding AMPs generally target highly conserved and essential components of microbial cellular structures, pathogens and commensals alike have developed strategies for surviving or evading the activities of AMPs. Mechanisms that result in the development of resistance involve modifications of outer cell wall components, such as LPS, teichoic acids, or phosphocholine, and the modulation of efflux pumps.

Under environmental stress, various microorganisms (such as *P. aeruginosa*, *Burkholderia cepacia* and *Burkholderia pseudomallei*, and non-typeable *Haemophilus influenzae*) have been shown to respond with signal transduction pathways that cause LPS modifications that decrease binding and killing by AMPs [102]. Additionally, Gram-positive bacteria may modify teichoic and lipoteichoic acid structure to decrease cell wall negative charges, thus reducing AMP binding [103].

Many organisms that colonize the respiratory tract can decorate their cell surfaces with host-derived phosphorylcholine. This form of host-mimicry results in reduced sensitivity to AMPs, and is exhibited by *Streptococcus pneumoniae*, *H. influenzae*, *Mycoplasma* and others [104].

Pathogens and commensals of the respiratory tract are often highly proteolytic. Microbial proteinases contribute to nutrient acquisition, tissue destruction and deregulation of inflammatory responses [105]. Proteolytic activity may contrast antimicrobial activity of respiratory secretions by degrading AMPs. Proteolytic degradation of the cathelicidin LL-37 has been demonstrated for *Streptococcus pyogenes* and *P. aeruginosa* [106].

AMPs as a Target in Host-Pathogen Interactions

It is becoming increasingly clear that AMPs exert complex functions within respiratory secretions such as antimicrobial activity, toxin-binding activity, chemotaxis, modulation of inflammatory responses, and activation of adaptive immunity. Several diseases in humans and laboratory animals are characterized by impairment in the function of an antimicrobial peptide [68]. Antimicrobial peptides qualify as prototypes of innovative drugs that might be used as antibiotics, antipolysaccharide drugs or modifiers of inflammation. Antibiotics based on these naturally occurring mammalian peptides might elicit fewer allergic reactions compared to conventional antibiotics. As these peptides serve as frontline defenses in respiratory secretions, administration of therapeutic candidates by the inhaled route is particularly appealing in respiratory diseases. Clinical trials using a porcine cathelicidin (protegrin) are currently underway [107]. Alternative strategies have also been attempted by administering gene therapy directly to the respiratory epithelium or systemically [108, 109]. In addition, increasing information is accumulating on the escape mechanisms through which bacteria modulate their anionic cell envelope contents (e.g. LPS and teichoic acids) and secrete inactivating proteases in order to acquire resistance to AMP. Knowledge of these modulating mechanisms may lead to the development of inhibitors capable of overriding bacterial attempts at acquiring resistance to antimicrobial peptides [110].

'Systemic' Antimicrobial Peptides

A number of peptides produced by different organ systems have been identified that exert antimicrobial properties.

Hepcidin. Hepcidins form a new gene family of immune-inducible, liver-expressed, cysteine-rich peptides. Human hepcidin, a 25-amino-acid peptide made by hepatocytes and also known as liver-expressed antimicrobial peptide (LEAP-1), is an antimicrobial peptide with iron-regulatory properties [111]. Compared with other antimicrobial peptides whose sequences have evolved

rapidly and vary significantly even between closely related mammalian species, the evolution of hepcidin is fairly constant among animal species. Synthetic hepcidin has shown to be active in vitro against Gram-negative pathogens and fungi, but not against key Gram-positive pathogens [112]. Hepcidin gene expression in the liver increases significantly within hours of infection with Gram-positive or Gram-negative pathogens, and infusion of IL-6 in human healthy volunteers [113].

Indolicidin. Indolicidin is a 13-residue antimicrobial peptide amide isolated from the cytoplasmic granules of bovine neutrophils. Indolicidin is active against a wide range of microorganisms and has also been shown to be hemolytic and cytotoxic towards erythrocytes and human T lymphocytes [114]. A recent study indicates that modified analogues of indolicidin exert potent antimicrobial activity and may become promising lead structures for developing future therapeutics [115].

Neuropeptides. In addition to catecholamines, secretory granules from chromaffin cells of the adrenal medulla contain neuropeptides derived from chromogranin A and proenkephalin A, which are released into the circulation in response to stress stimulation and exert antibacterial and antifungal activities. Among these, vasostatin-1 [116] and enkelytin [117] have also been identified in human polymorphonuclear secretions. Vasostatin-1 is strongly active against a variety of filamentous fungi, including *Aspergillus fumigatus* and *Fusarium oxysporum*, and pathogenic yeasts such as *C. albicans* at micromolar concentrations. Vasostatin-1 concentrations increase in infectious fluids following neutrophil release. Enkelytin displays potent antibacterial activity against Gram-positive bacteria including *Staphylococcus aureus* [117]. Ubifungin is a peptide found in all eukaryotic cells. This peptide inhibits the growth of filamentous fungi such as *A. fumigatus* and *F. oxysporum*, in addition to pathogenic yeasts, including *C. albicans* [118].

Vasostatin-1, enkelytin and ubifungin may represent promising non-toxic antimicrobial agents. They may be employed together or in combination with classical antibacterial and antifungal compounds to increase their efficacy [119].

Surfactant Proteins and Host Defense

Given the extent of the alveolar surface, it is of utmost importance that this district be equipped with mechanisms capable of actively contrasting invading inhaled microorganisms. Alveoli are lined by a surfactant layer that is mostly composed of surface-active phospholipids and a small protein component. Surfactant proteins A

and D (SP-A and SP-D) are members of the collectin family, which includes MBL, that map to the long arm of chromosome 10 [120]. These surfactant proteins are now considered to be involved in the clearance of microbes from the lung and to exert immunomodulatory functions. Pulmonary collectins bind and aggregate bacteria, fungi, viruses, and mycobacteria. Compared to other collectins such as MBL, SP-A and SP-D do not activate the complement cascade but rather coat microorganisms thus increasing phagocyte uptake, and also directly enhance macrophage phagocytosis and killing of invading organisms [120]. Surfactant collectins increase membrane permeability by as yet unknown mechanisms. Protein levels of both SP-A and SP-D increase in the alveolar compartment following pulmonary infections with microorganisms, suggesting that these proteins may act as analogues of acute-phase reactants in the lung [121]. Similarly to MBL, SP-A is composed of 18 subunit oligomers arranged in a 'flower bouquet' pattern, whereas SP-D forms a cruciform structure in the aqueous surfactant phase. It has recently been proposed that nucleic acids may be a novel class of ligands for collectins. SP-D binds to both DNA and RNA effectively and enhances the uptake of DNA by human monocytic cells [122]. This class of innate immune proteins could enhance the clearance of DNA that is released by necrotic cells or microbial cells.

Surfactant Protein A

SP-A is produced by alveolar type II cells, Clara cells, non-ciliated bronchiolar cells of the distal respiratory epithelium and by tracheobronchial glands [123]. SP-A ligands include microbial cell wall components such as the lipid A moiety of LPS and capsular polysaccharides, in addition to host cell receptors such as macrophage scavenger receptors. This pulmonary collectin binds to different bacterial strains of *S. pneumoniae*, *S. aureus*, *H. influenzae*, and *P. aeruginosa*, in addition to viruses such as *Herpes simplex* and respiratory syncytial virus, and organisms such as *Pneumocystis carinii* [124].

Genetic engineering techniques have led to the development of SP-A-deficient knockout mice, thus considerably enhancing our knowledge of the key functions of this surfactant protein [125]. Following tracheal instillation of different pathogens, compared to wild-type mice, SP-A-deficient animals showed decreased clearance of Gram-positive [126] and Gram-negative bacteria [127], viruses [128] and mycobacteria [129]. Defective microbial clearance has been traced to decreased macrophage microbial uptake and ingestion, coupled with reduced production of reactive oxygen and nitrogen species, resulting in in-

creased infection spread. Tissue inflammatory cell responses (neutrophil infiltration) and presence of proinflammatory cytokines, e.g. TNF- α , IL-6, and macrophage inflammatory protein-2, were greater in protein-defective mice compared to normal counterparts [130].

Taken together, these data indicate that SP-A plays a role in activating alveolar clearance of numerous respiratory pathogens, enhancing macrophage binding and uptake, increasing the production of microbicidal free radicals and reducing proinflammatory cytokine release. These activities promote pathogen phagocytosis and killing, whilst dampening excessive inflammatory activation that would lead to tissue damage in the delicate alveolar environment.

Surfactant Protein D

SP-D was first identified as a constituent of surfactant alveolar cells, but is also present in a variety of different human tissues such as pancreatic ducts, bile ducts, sweat glands, parotid and lacrimal glands [131]. It is expressed primarily by type II alveolar cells and serous cells in tracheobronchial tree glands. Similarly to SP-A, SP-D also displays the capacity to bind to a vast array of Gram-positive and Gram-negative bacteria, viruses, fungi, yeast, and mycobacteria. However, SP-D shows a greater propensity to aggregate following pathogen binding, thus favoring receptor-mediated opsonization and phagocytosis. Unlike SP-A, SP-D does not bind to the lipid A component of LPS but to the core oligosaccharide [132].

Null mutations of the SP-D genetic locus have been developed in mice. Experimental infection of these animals has shown that SP-D is involved in the clearance of viral but not bacterial pathogens [124]. SP-D enhances macrophage phagocytosis of *P. aeruginosa* strains, and neutrophil uptake of Gram-positive and Gram-negative organisms [133, 134]. Contrary to SP-A, SP-D reduces rather than augments macrophage oxygen radical production [130]. Data from animal models indicate that SP-D exerts a dampening effect on excessive inflammatory activation following infection by reducing total inflammatory cell counts and proinflammatory cytokine levels [130].

Surfactant Collectins and Host-Pathogen Interactions

Although much work has been performed to elucidate the interplay between surfactant collectins and invading organisms, further information is certainly welcome. The role of collectins in reducing proinflammatory stimuli following infection is still a matter of controversy. A recent

study proposes that SP-A and SP-D may modulate inflammatory mediator production based on differential binding. Activation of the transmembrane signal-regulating protein α (SIRP α) may block proinflammatory signal production, whereas calreticulin/CD91 binding stimulates proinflammatory mediator synthesis [135].

It remains to be explained why binding of SP-D to certain bacteria results in increased phagocytosis by alveolar macrophages, whereas binding to *M. tuberculosis* has the opposite effect [136]. Some microorganisms may increase the efficacy of infection by using SP-A as a means to gain entry into target cells [137]. Furthermore, it is now being recognized that bacteria have developed ways of eluding collectin bactericidal activity by modifying LPS ligand moieties [138]. A better understanding of collectin-mediated immunity may in future allow the identification of disease states in which the therapeutic administration of collectins may be beneficial [139].

Conclusions

That the extensive alveolar surface area is kept sterile at most times, notwithstanding the constant burden of toxins, antigens, and microbes it is exposed to, is an outstanding achievement of the organism's defense system. The traditional view was that this feat was made possible through the help of cough, mucociliary clearance, epithelial barrier function, and alveolar macrophages.

Over the last decade, our understanding of innate immune defenses has added an impressive amount of new actors to the scene. The airway epithelium is no longer considered a passive barrier to microbial invasion, it is now rather interpreted as an immune organ with the ca-

pabilities of detecting microorganisms and inducing inflammatory and host defense responses. Soluble and membrane-based receptors have been identified that are capable of recognizing non-mammalian microbial motifs and mobilizing close to instantaneous inflammatory reactions towards infection. An increasing number of peptides exerting antimicrobial activity are being recognized in airway secretions, associated with chemokines, cytokines, proteinase inhibitors, and surfactant proteins.

Interestingly, genetic polymorphisms associated with reduced production or activity of these factors have been recognized as potential modifiers in the natural history of different respiratory diseases. Better knowledge of underlying genetic traits may help identify patients at greater risk of severe complications following infection. On the other hand, overactivation of innate immune responses may be associated with excessive inflammation and tissue damage such as may be observed in sepsis.

In the future it may be possible to modulate the innate immune response in order to downplay pathways leading to tissue inflammation while enhancing mechanisms involved in microbial elimination. Furthermore, synthetic analogues of antimicrobial peptides may evolve into novel and independent classes of antibiotics. Current antibiotic treatment of infections is plagued by the rising phenomenon of microbial resistance among important pathogens, an uneven supply of novel antibiotic classes, and a reduction in pharmaceutical companies investing in anti-infective agents. In view of the considerable burden in terms of mortality and morbidity that severe infections still pose worldwide, a better understanding of the biological basis of host-pathogen interactions opens stimulating future treatment perspectives.

References

- 1 Medzhitov R, Janeway CA: Innate immunity: Impact on the adaptive immune response. *Curr Opin Immunol* 1997;9:4–9.
- 2 Delves PJ, Roitt IM: The immune system. *N Engl J Med* 2000;343:37–49.
- 3 Medzhitov R, Janeway CA: Innate immunity. *N Engl J Med* 2000;343:338–344.
- 4 Turner MW, Hamvas RMJ: Mannose-binding lectin: Structure, function, genetics and disease associations. *Rev Immunogenet* 2002;2:305–322.
- 5 Matsushita M, Thiel S, Jensenius JC, Terai I, Fujita T: Proteolytic activities of two types of mannose-binding lectin-associated serine protease. *J Immunol* 2000;165:2637–2642.
- 6 Madsen HO, Garred P, Kurtzhals JAL, Lamm LU, Ryder LP, Thiel S, Svejgaard A: A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. *Immunogenetics* 1994;40:37–44.
- 7 Madsen HO, Garred P, Thiel S, Kurtzhals JA, Lamm LU, Ryder LP, Svejgaard A: Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein *J Immunol* 1995;155:3013–3020.
- 8 Koch A, Melbye M, Sorensen P, Homoe P, Madsen HO, Molbak K, Hansen CH, Andersen LH, Hahn GW, Garred P: Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. *JAMA* 2001;285:1316–1321.
- 9 Davies JC, Turner MW, Klein N: Impaired pulmonary status in cystic fibrosis adults with two mutated *MBL-2* alleles. *Eur Respir J* 2004; 24:798–804.
- 10 Roy S, Knox K, Segal S, Griffiths D, Moore CE, Welsh KI, Smarason A, Day NP, McPheat WL, Crook DW, Hill AV: *MBL* genotype and risk of invasive pneumococcal disease: A case-control study. *Lancet* 2002;359:1569–1573.

- 11 Hoal-Van Helden EG, Epstein J, Victor TC, Hon D, Lewis LA, Beyers N, Zurakowski D, Ezekowitz AB, Van Helden PD: Mannose-binding protein B allele confers protection against tuberculous meningitis. *Pediatr Res* 1999;45:459-464.
- 12 Garred P, Pressler T, Lanng S, Madsen HO, Moser C, Laursen I, Balstrup F, Koch C, Koch C: Mannose-binding lectin (MBL) therapy in an MBL-deficient patient with severe cystic fibrosis lung disease. *Pediatr Pulmonol* 2002;33:201-207.
- 13 Fraser IP, Koziel H, Ezekowitz A: The serum mannose-binding protein and the macrophage mannose receptor are pattern recognition molecules that link innate and adaptive immunity. *Semin Immunol* 1998;10:363-372.
- 14 Thomas CA, Li Y, Kodama T, Suzuki H, Silverstein SC, El Khoury J: Protection from lethal gram-positive infection by macrophage scavenger receptor-dependent phagocytosis. *J Exp Med* 2000;191:147-156.
- 15 Savill J: Recognition and phagocytosis of cells undergoing apoptosis. *Br Med Bull* 1997;53:491-508.
- 16 Anderson KV, Jurgens G, Nusslein-Volhard C: Establishment of dorsal-ventral polarity in the *Drosophila* embryo: Genetic studies on the role of the Toll gene product. *Cell* 1985;42:779-789.
- 17 Lemaitre BE, Nicolas E, Michaut NL, Reichhart JM, Hoffmann JA: The dorsoventral regulatory gene cassette *spatzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults. *Cell* 1996;86:973-983.
- 18 O'Neill LA, Greene C: Signal transduction pathways activated by the IL-1 receptor family: Ancient signaling machinery in mammals, insects, and plants. *J Leukoc Biol* 1998;63:650-657.
- 19 Lien E, Sellati TJ, Yoshimura A, Flo TH, Rawadi G, Finberg RW, Carroll JD, Espevik T, Ingalls RR, Radolf JD, Golenbock DT: Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. *J Biol Chem* 1999;274:33419-33425.
- 20 Kadowaki N, Ho S, Antonenko S, Malefyt RW, Kastelein RA, Liu YJ: Subsets of human dendritic cell precursors express different Toll-like receptors and respond to different microbial antigens. *J Exp Med* 2001;194:863-869.
- 21 Alexopoulou L, Holt AC, Medzhitov R, Flavell RA: Recognition of double-stranded RNA and activation of NF- κ B by Toll-like receptor 3. *Nature* 2001;413:732-738.
- 22 Beutler B: Tlr4: Central component of the sole mammalian LPS sensor. *Curr Opin Immunol* 2000;12:20-26.
- 23 Ohashi K, Burkart V, Flohe S, Kolb H: Heat shock protein 60 is a putative endogenous ligand of the Toll-like receptor-4 complex. *J Immunol* 2000;164:558-561.
- 24 Kurt-Jones EA, Popova L, Kwinn L, Haynes LM, Jones LP, Tripp RA, Walsh EE, Freeman MW, Golenbock DT, Anderson LJ, Finberg RW: Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat Immunol* 2000;1:398-401.
- 25 Fenton MJ, Golenbock DT: LPS-binding proteins and receptors. *J Leukoc Biol* 1998;64:25-32.
- 26 Kitchens RL, Thompson PA, Viriyakosol S, O'Keefe GE, Munford RS: Plasma CD14 decreases monocyte response to LPS by transferring cell-bound LPS to plasma lipoproteins. *J Clin Invest* 2001;108:485-493.
- 27 Miyake K: Innate recognition of lipopolysaccharide by Toll-like receptor 4-MD-2. *Trends Microbiol* 2004;12:186-192.
- 28 Nagai Y, Akashi S, Nagafuku M, Ogata M, Iwakura Y, Akira S, Kitamura T, Kosugi A, Kimoto M, Miyake K: Essential role of MD-2 in LPS responsiveness and TLR4 distribution. *Nat Immunol* 2002;3:667-672.
- 29 Ohnishi T, Muroi M, Tanamoto K: MD-2 is necessary for the toll-like receptor 4 protein to undergo glycosylation essential for its translocation to the cell surface. *Clin Diagn Lab Immunol* 2003;10:405-410.
- 30 Akashi S, Nagai Y, Ogata H, Oikawa M, Fukase K, Kusumoto S, Kawasaki K, Nishijima M, Hayashi S, Kimoto M, Miyake K: Human MD-2 confers on mouse toll-like receptor 4 species-specific lipopolysaccharide recognition. *Int Immunol* 2001;13:1595-1599.
- 31 Viriyakosol S, Tobias PS, Kitchens RL, Kirkland TN: MD-2 binds to bacterial lipopolysaccharide. *J Biol Chem* 2001;276:38044-38051.
- 32 Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A: The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 2001;420:1099-1103.
- 33 Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, Akira S: A Toll-like receptor recognizes bacterial DNA. *Nature* 2000;408:740-744.
- 34 Takeuchi O, Kawai T, Muhlratt PF, Morr M, Radolf JD, Zychlinsky A, Takeda K, Akira S: Discrimination of bacterial lipoproteins by Toll-like receptor 6. *Int Immunol* 2001;13:933-940.
- 35 Yamamoto M, Sato S, Hemmi H, Sanjo H, Uematsu S, Kaisho T, Hoshino K, Takeuchi O, Kobayashi M, Fujita T, Takeda K, Akira S: Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4. *Nature* 2002;420:324-329.
- 36 Wesche H, Henzel WJ, Shillinglaw W, Li S, Cao Z: MyD88: An adapter that recruits IRAK to the IL-1 receptor complex. *Immunity* 1997;7:837-847.
- 37 Suzuki N, Suzuki S, Duncan GS, Millar DG, Wada T, Mirtsos C, Takada H, Wakeham A, Itie A, Li S, Penninger JM, Wesche H, Ohashi PS, Mak TW, Yeh WC: Severe impairment of interleukin-1 and Toll-like receptor signaling in mice lacking IRAK-4. *Nature* 2002;416:750-756.
- 38 Kobayashi K, Hernandez LD, Galan JE, Janeway CA, Medzhitov R, Flavell RA: IRAK-M is a negative regulator of Toll-like receptor signaling. *Cell* 2002;110:191-202.
- 39 O'Neill LA: Signal transduction pathways activated by the IL-1 receptor/Toll-like receptor superfamily. *Curr Top Microbiol Immunol* 2002;270:17-61.
- 40 Zhang G, Ghosh S: Toll-like receptor-mediated NF- κ B activation: A phylogenetically conserved paradigm in innate immunity. *J Clin Invest* 2001;107:13-19.
- 41 Diamond G, Kaiser V, Rhodes J, Russell JP, Bevins CL: Transcriptional regulation of beta-defensin gene expression in tracheal epithelial cells. *Infect Immun* 2000;68:113-119.
- 42 Karin M, Ben-Neriah Y: Phosphorylation meets ubiquitination: The control of NF- κ B activity. *Annu Rev Immunol* 2000;18:621-663.
- 43 Kopp E, Medzhitov R, Carothers J, Xiao C, Douglas I, Janeway CA, Ghosh S: ECSIT is an evolutionarily conserved intermediate in the Toll/IL-1 signal transduction pathway. *Genes Dev* 1999;13:2059-2071.
- 44 Chang L, Karin M: Mammalian MAP kinase signaling cascades. *Nature* 2001;420:37-40.
- 45 Yamamoto M, Sato S, Hemmi H, Uematsu S, Hoshino K, Kaisho T, Takeuchi O, Takeda K, Akira S: TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. *Nat Immunol* 2003;4:1144-1150.
- 46 Cohen J: The immunopathogenesis of sepsis. *Nature* 2002;420:885-891.
- 47 Agrawal A: Different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos. *J Immunol* 2003;171:4984-4989.
- 48 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;197:403-411.
- 49 Aliprantis AO, Yang RB, Mark MR, Suggestt S, Devaux B, Radolf JD, Klimpel GR, Godowski P, Zychlinsky A: Cell activation and apoptosis by bacterial lipoproteins through Toll-like receptor-2. *Science* 1999;285:736-739.
- 50 Netea MG, Van der Meer JW, Kullberg BJ: Toll-like receptors as an escape mechanism from the host defense. *Trends Microbiol* 2004;12:484-488.
- 51 Hashimoto M, Asai Y, Ogawa T: Treponemal phospholipids inhibit innate immune responses by pathogen-associated molecular patterns. *J Biol Chem* 2003;278:44205-44213.
- 52 Harte MT, Haga IR, Maloney G, Gray P, Reading PC, Bartlett NW, Smith GL, Bowie A, O'Neill LA: The poxvirus protein A52R targets Toll-like receptor signaling complexes to suppress host defense. *J Exp Med* 2003;197:343-350.
- 53 Gewirtz AT, Yu Y, Krishna US, Israel DA, Lyons SL, Peek RM: *Helicobacter pylori* flagellin evades Toll-like receptor 5-mediated innate immunity. *J Infect Dis* 2004;189:1914-1920.

- 54 Sundstrom JB, Little DM, Villinger F, Ellis JE, Ansari AA: Signaling through Toll-like receptors triggers HIV-1 replication in latently infected mast cells. *J Immunol* 2004;172:4391-4399.
- 55 Iwami K, Matsuguchi T, Masada A, Kikuchi T, Musikacharoen T, Yoshikai Y: Cutting edge: Naturally occurring soluble form of mouse Toll-like receptor 4 inhibits lipopolysaccharide signaling. *J Immunol* 2001;165:6682-6686.
- 56 Means TK, Golenbock DT, Fenton MJ: The biology of Toll-like receptors. *Cytokine Growth Factor Rev* 2000;11:219-232.
- 57 Yeh WC, Chen NJ: Another Toll road. *Nature* 2003;424:736-737.
- 58 Gabay C, Kushner I: Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999;340:448-454.
- 59 Thompson D, Pepys MB, Wood SP: The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure* 1999;7:169-177.
- 60 Pepys MB, Hirschfield GM: C-reactive protein: A critical update. *J Clin Invest* 2003;111:1805-1812.
- 61 Knowles MR, Boucher RC: Mucus clearance as a primary innate defense mechanism for mammalian airways. *J Clin Invest* 2002;109:571-577.
- 62 Tarran R, Grubb BR, Gatzky JT, Davis CW, Boucher RC: The relative roles of passive surface forces and active ion transport in the modulation of airway surface liquid volume and composition. *J Gen Physiol* 2001;118:223-236.
- 63 Worlitzsch D, Tarran R, Ulrich M, Schwab U, Cekici A, Meyer KC, Birrer P, Bellon G, Berger J, Weiss T, Botzenhart K, Yankaskas JR, Randell S, Boucher RC, Doring G: Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. *J Clin Invest* 2002;109:317-325.
- 64 Bennett WD, Olivier KN, Zeman KL, Hohnaker KW, Boucher RC, Knowles MR: Effect of uridine 5-triphosphate plus amiloride on mucociliary clearance in adult cystic fibrosis. *Am J Respir Crit Care Med* 1996;153:1796-1801.
- 65 Zeitlin PL, Diener-West M, Rubinstein RC, Boyle MP, Lee CK, Brass-Ernst L: Evidence of CFTR function in cystic fibrosis after systemic administration of 4-phenylbutyrate. *Mol Ther* 2002;6:119-126.
- 66 Egan ME, Pearson M, Weiner SA, Rajendran V, Rubin D, Glockner-Pagel J, Canny S, Du K, Lukacs GL, Caplan MJ: Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. *Science* 2004;304:600-602.
- 67 Wu H, Song Z, Hentzer M, Andersen JB, Molin S, Givskov M, Hoiby N: Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in *Pseudomonas aeruginosa* lung infection in mice. *J Antimicrob Chemother* 2004;53:1054-1061.
- 68 Zasloff M: Antimicrobial peptides of multicellular organisms. *Nature* 2002;415:389-395.
- 69 Bals R: Epithelial antimicrobial peptides in host defense against infection. *Respir Res* 2000;1:141-150.
- 70 Ganz T: Epithelia: Not just physical barriers. *Proc Natl Acad Sci USA* 2002;99:3357-3358.
- 71 Nagaoka I, Hirota S, Yomogida S, Ohwada A, Hirata M: Synergistic actions of antibacterial neutrophil defensins and cathelicidins. *Inflamm Res* 2000;49:73-79.
- 72 Thompson AB, Bohling T, Payvandi F, Renard SI: Lower respiratory tract lactoferrin and lysozyme arise primarily in the airways and are elevated in association with chronic bronchitis. *J Lab Clin Med* 1990;115:148-158.
- 73 Ganz T: Antimicrobial polypeptides in host defense of the respiratory tract. *J Clin Invest* 2002;109:693-697.
- 74 Orsi N: The antimicrobial activity of lactoferrin: Current status and perspectives. *Biomaterials* 2004;17:189-196.
- 75 Zhu J, Nathan C, Ding A: Suppression of macrophage responses to bacterial lipopolysaccharide by a non-secretory form of secretory leukocyte protease inhibitor. *Biochim Biophys Acta* 1999;1451:219-223.
- 76 Simpson AJ, Maxwell AI, Govan JRW, Haslett C, Sallenave JM: Elafin (elastase-specific inhibitor) has anti-microbial activity against Gram-positive and Gram-negative respiratory pathogens. *FEBS Lett* 1999;452:309-313.
- 77 Canny G, Levy O, Furuta GT, Narravula-Aliapati S, Sisson RB, Serhan CN, Colgan SP: Lipid mediator-induced expression of bactericidal/permeability-increasing protein (BPI) in human mucosal epithelia. *Proc Natl Acad Sci USA* 2002;99:3902-3907.
- 78 Levy O: Therapeutic potential of the bactericidal/permeability-increasing protein. *Expert Opin Investig Drugs* 2002;11:159-167.
- 79 Andraut JB, Gaillard I, Giorgi D, Rouquier S: Expansion of the BPI family by duplication on human chromosome 20: Characterization of the RY gene cluster in 20q11.21 encoding olfactory transporters/antimicrobial-like peptides. *Genomics* 2003;82:172-184.
- 80 Bingle CD, Gorr SU: Host defense in oral and airway epithelia: Chromosome 20 contributes a new protein family. *Int J Biochem Cell Biol* 2004;36:2144-2152.
- 81 Lehrer R, Ganz T, Selsted M: Defensins: Endogenous antibiotic peptides of animal cells. *Cell* 1991;64:229-230.
- 82 Linzmeier R, Ho CH, Hoang BV, Ganz T: A 450-kb contig of defensin genes on human chromosome 8p23. *Gene* 1999;233:205-211.
- 83 Soong L, Ganz T, Ellison A, Caughey G: Purification and characterization of defensins from cystic fibrosis sputum. *Inflamm Res* 1997;46:98-102.
- 84 McCray P Jr, Bentley L: Human airway epithelia express a β -defensin. *Am J Respir Cell Mol Biol* 1997;16:343-349.
- 85 Becker MN, Diamond G, Verghese MW, Randell SH: CD14-dependent lipopolysaccharide-induced beta-defensin-2 expression in human tracheobronchial epithelium. *J Biol Chem* 2000;275:29731-29736.
- 86 Singh PK, Jia HP, Wiles K, Hesselberth J, Liu L, Conway BA, Greenberg EP, Valore EV, Welsh MJ, Ganz T, Tack BF, McCray PB Jr: Production of β -defensins by human airway epithelia. *Proc Natl Acad Sci USA* 1998;95:14961-14966.
- 87 Daulebaev N, Gropp R, Frye M, Loitsch S, Wagner TO, Bargon J: Expression of human β -defensin (HBD-1 and HBD-2) mRNA in nasal epithelia of adult cystic fibrosis patients, healthy individuals and individuals with acute colds. *Respiration* 2002;69:46-51.
- 88 Zanetti M, Gennaro R, Romeo D: Cathelicidins: A novel protein family with a common proregion and a variable C-terminal antimicrobial domain. *FEBS Lett* 1995;374:1-5.
- 89 Bals R, Wang X, Zasloff M, Wilson JM: The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. *Proc Natl Acad Sci USA* 1998;95:9541-9546.
- 90 Schaller-Bals S, Schulze A, Bals R: Increased levels of antimicrobial peptides in tracheal aspirates of newborn infants during infection. *Am J Respir Crit Care Med* 2002;165:992-995.
- 91 Bals R, Weiner DJ, Moscioni AD, Meegalla RL, Wilson JM: Augmentation of innate host defence by expression of a cathelicidin antimicrobial peptide. *Infect Immun* 1999;67:6084-6089.
- 92 van der Spek JC, Offner GD, Troxler RF, Oppenheim FG: Molecular cloning of human submandibular histatins. *Arch Oral Biol* 1990;35:137-143.
- 93 Gusman H, Travis J, Helmerhorst EJ, Potempa J, Troxler RF, Oppenheim FG: Salivary histatin 5 is an inhibitor of both host and bacterial enzymes implicated in periodontal disease. *Infect Immun* 2001;69:1402-1408.
- 94 Yang D, Biragyn A, Kwak LW, Oppenheim JJ: Mammalian defensins in immunity: More than just microbicidal. *Trends Immunol* 2002;23:291-296.
- 95 Van Wetering S, Mannesse-Lazeroms SP, Van Sterkenburg MA, Daha MR, Dijkman JH, Hiemstra PS: Effect of defensins on interleukin-8 synthesis in airway epithelial cells. *Am J Physiol* 1997;272:L888-L896.
- 96 Van Wetering S, van der Linden AC, van Sterkenburg MA, de Boer WI, Kuijpers AL, Schalkwijk J, Hiemstra PS: Regulation of SLPI and elafin release from bronchial epithelial cells by neutrophil defensins. *Am J Physiol Lung Cell Mol Physiol* 2000;278:L51-58.
- 97 Van Wetering S, Rahman I, Hiemstra PS, MacNee W: Role of intracellular glutathione in neutrophil defensin-induced IL-8 synthesis and cytotoxicity in airway epithelial cells. *Eur Respir J* 1998;12:420s.
- 98 Aarbiou J, Ertmann M, van Wetering S, van Noort P, Rook D, Rabe KF, Litvinov SV, van Krieken JH, de Boer WI, Hiemstra PS: Human neutrophil defensins induce lung epithelial cell proliferation in vitro. *J Leukoc Biol* 2002;72:167-174.

- 99 Ganz T, Lehrer RI: Defensins. *Pharmacol Ther* 1995;66:191–205.
- 100 Gennaro R, Zanetti M: Structural features and biological activities of the cathelicidin-derived antimicrobial peptides. *Biopolymers* 2000;55:31–49.
- 101 Yang D, Chertov O, Bykowska S, Chen Q, Buffo M, Shogan J, Anderson M, Schroder J, Wang J, Howard O, Oppenheim J: β -Defensins: Linking innate and adaptive immunity through dendritic and T cell CCR6. *Science* 1999;286:525–528.
- 102 Devine DA: Antimicrobial peptides in defence of the oral and respiratory tracts. *Mol Immunol* 2003;40:431–443.
- 103 Peschel A: How do bacteria resist human antimicrobial peptides? *Trends Microbiol* 2002;10:179–186.
- 104 Lysenko ES, Gould J, Bals R, Wilson JM, Weiser JN: Bacterial phosphorylcholine decreases susceptibility to the antimicrobial peptide LL-37/hCAP18 expressed in the upper respiratory tract. *Infect Immun* 2000;68:1664–1671.
- 105 Potempa J, Babbula A, Travis J: Role of bacterial proteinases in matrix destruction and modulation of host responses. *Periodontology* 2000;24:153–192.
- 106 Schmidtchen A, Frick IM, Andersson E, Tapper H, Björk L: Proteinases of common pathogenic bacteria degrade and inactivate the antibacterial peptide LL-37. *Mol Microbiol* 2002;46:157–168.
- 107 Ganz T, Bellm L, Lehrer RI: Protegrins: New antibiotics of mammalian origin. *Expert Opin Investig Drugs* 2000;9:1731–1742.
- 108 Bals R, Weiner DJ, Moscioni AD, Meegalla RL, Wilson JM: Augmentation of innate host defense by expression of a cathelicidin antimicrobial peptide. *Infect Immun* 1999;67:6084–89.
- 109 Bals R, Weiner DJ, Meegalla RL, Wilson JM: Transfer of a cathelicidin peptide antibiotic gene restores bacterial killing in a cystic fibrosis xenograft model. *J Clin Invest* 1999;103:1113–1117.
- 110 Weidenmaier C, Kristian SA, Peschel A: Bacterial resistance to antimicrobial host defenses: An emerging target for novel anti-infective strategies? *Curr Drug Targets* 2003;4:643–649.
- 111 Park CH, Valore EV, Waring AJ, Ganz T: Hepcidin, a urinary antimicrobial peptide synthesized in the liver. *J Biol Chem* 2001;276:7806–7810.
- 112 Lauth X, Babon JJ, Stannard JA, Singh S, Nizet V, Carlberg JM, Ostland VE, Pennington MW, Norton RS, Westerman ME: Bass hepcidin: Synthesis, solution structure, antimicrobial activities and synergism, and in vivo hepatic response to bacterial infections. *J Biol Chem* 2004 [Epub ahead of print].
- 113 Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, Ganz T: IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest* 2004;113:1271–1276.
- 114 Subbalakshmi C, Sitaram N: Mechanism of antimicrobial action of indolicidin. *FEMS Microbiol Lett* 1998;160:91–96.
- 115 Ryge TS, Doisy X, Ifrah D, Olsen JE, Hansen PR: New indolicidin analogues with potent antibacterial activity. *J Pept Res* 2004;64:171–185.
- 116 Lugardon K, Raffner R, Goumon Y, Corti A, Delmas A, Bulet P, Aunis D, Metz-Boutigue MH: Antibacterial and antifungal activities of vasostatin-I, the N-terminal fragment of chromogranin A. *J Biol Chem* 2000;275:10745–10753.
- 117 Goumon Y, Lugardon K, Kieffer B, Lefevre JF, Van Dorsselaer A, Aunis D, Metz-Boutigue MH: Characterization of antibacterial COOH-terminal proenkephalin-A-derived peptides (PEAP) in infectious fluids. Importance of enkelytin, the antibacterial PEAP209–237 secreted by stimulated chromaffin cells. *J Biol Chem* 1998;273:29847–29856.
- 118 Kieffer AE, Goumon Y, Ruh O, Chasserot-Golaz S, Nullans G, Gasnier C, Aunis D, Metz-Boutigue MH: The N- and C-terminal fragments of ubiquitin are important for antimicrobial activities. *FASEB J* 2003;17:776–778.
- 119 Metz-Boutigue MH, Kieffer AE, Goumon Y, Aunis D: Innate immunity: Involvement of new neuro-peptides. *Trends Microbiol* 2003;11:585–592.
- 120 Wright JR: Immunomodulatory functions of surfactant. *Physiol Rev* 1997;77:931–962.
- 121 Atochina EN, Beck JM, Scanlon ST, Preston AM, Beers MF: *Pneumocystis carinii* pneumonia alters expression and distribution of lung collectins SP-A and SP-D. *J Lab Clin Med* 2001;137:429–439.
- 122 Palaniyar N, Nadesalingam J, Reid KB: Innate immune collectins bind nucleic acids and enhance DNA clearance in vitro. *Ann NY Acad Sci* 2003;1010:467–470.
- 123 Khoor A, Gray ME, Hull WM, Whitsett JA, Stahlman MT: Developmental expression of SP-A and SP-A mRNA in the proximal and distal respiratory epithelium in the human fetus and newborn. *J Histochem Cytochem* 1993;41:1311–1319.
- 124 Le Vine AM, Whitsett JA: Pulmonary collectins and innate host defense of the lung. *Microbes Infect* 2001;3:161–166.
- 125 Korfhagen TR, Bruno MD, Ross GF, Huelsman KM, Ikegami M, Jobe AH, Wert SE, Stripp BR, Morris RE, Glasser SW, Bachurski CJ, Iwamoto HS, Whitsett JA: Altered surfactant function and structure in SP-A gene targeted mice. *Proc Natl Acad Sci USA* 1996;93:9594–9599.
- 126 LeVine AM, Bruno MD, Huelsman KM, Ross GF, Whitsett JA, Korfhagen TR: Surfactant protein A-deficient mice are susceptible to group B streptococcal infection. *J Immunol* 1997;158:4336–4340.
- 127 LeVine AM, Kurak KE, Bruno MD, Stark JM, Whitsett JA, Korfhagen TR: Surfactant protein-A-deficient mice are susceptible to *Pseudomonas aeruginosa* infection. *Am J Respir Cell Mol Biol* 1998;19:700–708.
- 128 LeVine AM, Gwozdz J, Stark J, Bruno MD, Whitsett JA, Korfhagen TA: Surfactant protein-A enhances respiratory syncytial virus clearance in vivo. *J Clin Invest* 1999;103:1015–1021.
- 129 Gaynor CD, McCormack FX, Voelker DR, McGowan SE, Schlesinger LS: Pulmonary surfactant protein A mediates enhanced phagocytosis of *Mycobacterium tuberculosis* by a direct interaction with human macrophages. *J Immunol* 1995;155:5343–5351.
- 130 LeVine AM, Whitsett JA, Gwozdz JA, Richardson TR, Fisher JH, Burhans MS, Korfhagen TR: Distinct effects of surfactant protein A and D deficiency during bacterial infection on the lung. *J Immunol* 2000;165:3934–3940.
- 131 Madsen J, Kliem A, Tornoe I, Skjodt K, Koch C, Holmskov U: Localization of lung surfactant protein D on mucosal surfaces in human tissue. *J Immunol* 2000;164:5866–5870.
- 132 Van Iwaarden JF, Pikaar JC, Storm J, Brouwer E, Verhoef J, Oosting RS, van Golde LM, van Strijp JA: Binding of surfactant protein A to the lipid A moiety of bacterial lipopolysaccharides. *Biochem J* 1994;303:407–411.
- 133 Restrepo CI, Dong Q, Savov J, Mariencheck WI, Wright JR: Surfactant protein D stimulates phagocytosis of *Pseudomonas aeruginosa* by alveolar macrophages. *Am J Respir Cell Mol Biol* 1999;21:576–585.
- 134 Hartshorn KL, Crouch E, White MR, Colamussi ML, Kakkanatt A, Tauber B, Shepherd V, Sastry KN: Pulmonary surfactant proteins A and D enhance neutrophil uptake of bacteria. *Am J Physiol* 1998;274:L958–L969.
- 135 Gardai SJ, Xiao YQ, Dickinson M, Nick JA, Voelker DR, Greene KE, Henson PM: By binding SIRP α or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. *Cell* 2003;115:13–23.
- 136 Ferguson JS, Voelker DR, Ufnar JA, Dawson AJ, Schlesinger LS: Surfactant protein D inhibition of human macrophage uptake of *Mycobacterium tuberculosis* is independent of bacterial agglutination. *J Immunol* 2002;168:1309–1314.
- 137 Hickling TP, Malhotra R, Bright H, McDowell W, Blair ED, Sim RB: Lung surfactant protein A provides a route of entry for respiratory syncytial virus into host cells. *Viral Immunol* 2000;13:125–135.
- 138 Schaeffer LM, McCormack FX, Wu H, Weiss AA: Interactions of pulmonary collectins with *Bordetella bronchiseptica* and *Bordetella pertussis* lipopolysaccharide elucidate the structural basis of their antimicrobial activities. *Infect Immun* 2004;72:7124–7130.
- 139 van de Wetering JK, van Golde LM, Batenburg JJ: Collectins: Players of the innate immune system. *Eur J Biochem* 2004;271:1229–1249.