

## Innate immunity

*Innate immune mechanisms confer essential first-line host defence against the unremitting threat posed by environmental microbial and viral pathogens.*

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Mechanical barriers presented by the skin and mucus membranes, together with anti-infective chemicals and enzymes present on their surfaces, impede the access of potential pathogens to the epithelium and underlying tissues. If these are subverted, the interactions of invasive pathogens with various types of pathogen recognition receptors on epithelial cells and resident cells of the innate immune system, especially macrophages, initiate a localised inflammatory response characterised by an early influx of blood neutrophils.<sup>1,2</sup>

A comparison of the major characteristics of innate and adaptive immune responses, as well as the cellular elements involved, is shown in Table 1. It must be emphasised, however, that these two immune systems do not function in isolation, but rather cooperate

to provide optimum host defence. Although not listed in Table 1, epithelial cells and endothelial cells, and to a lesser extent other types of structural cells such as fibroblasts and smooth-muscle cells, are critically involved in promoting both innate and adaptive immune responses. In the case of epithelial cells, this is achieved via production of pro-inflammatory chemokines and cytokines, while endothelial cells orchestrate leukocyte transendothelial migration at sites of infection.<sup>1,2</sup>

The following aspects of innate immunity will be addressed in the remaining sections of this review:

- the barrier and expulsive functions of the epithelium, as well as enzymes and chemicals with broad-spectrum antimicrobial and/or antiviral activity present in epithelial lining fluid

- the inflammatory response initiated by pathogen/pathogen recognition receptor interactions
- optimisation of host defences through cooperation with adaptive immune mechanisms.

### The epithelium in innate immunity

Notwithstanding the involvement of epithelial pattern recognition receptors in triggering protective inflammatory responses, the epithelium functions as both a barrier and hostile environment.

#### The barrier function of the epithelium

The physical barriers presented by epithelial surfaces (skin, upper and lower respiratory tract, gastrointestinal tract, conjunctiva) represent a resilient first-line barrier to most infectious agents, which is complemented by several additional mechanisms including:

- the expulsive effects of the mucociliary escalator in the airways
- the flushing effects of saliva, tears and urine on epithelial surfaces
- peristalsis in the GIT, as well as the low pH of gastric fluid, antagonism by normal gut flora, and the antimicrobial actions of bile acids.<sup>1,2</sup>

#### Anti-infective mechanisms of epithelial lining fluid

Epithelial lining fluids contain high concentrations of the anti-infective proteins

**Table 1. Comparison of the properties of the innate and adaptive immune systems**

Innate immunity	Adaptive immunity
<ul style="list-style-type: none"><li>• First line in host defence</li><li>• Generally lacks specificity</li><li>• Generally lacks memory</li></ul>	<ul style="list-style-type: none"><li>• Second line in host defence</li><li>• Highly specific for a particular pathogen i.e. antigen-specific</li><li>• Possesses memory i.e. the onset of the response is faster and the magnitude greater with successive exposures to the pathogen</li><li>• Antigen-presenting cells, T lymphocytes and B lymphocytes are the major cellular components</li></ul>
<ul style="list-style-type: none"><li>• Dendritic cells, mast cells, monocytes/macrophages, natural killer (NK) cells, polymorphonuclear leukocytes (PMNL) and <math>\gamma\delta</math>T cells are primarily involved</li></ul>	

lysozyme, secretory phospholipase A<sub>2</sub>, the surfactant proteins A and D (SP-A and SP-D), as well as several types of anti-infective peptides. Lysozyme is a ubiquitous enzyme present in tears, saliva, mucus, gastric juice and human milk. It is produced by various types of cell including granulocyte precursors in the bone marrow, monocytes/macrophages, various exocrine glands, cartilage, and Paneth cells of the GIT.<sup>3</sup> It selectively targets the cell wall of gram-positive bacteria, hydrolysing the 1,4-beta linkage between N-acetylmuramic acid and N-acetyl-D-glucosamine. Secretory phospholipase A<sub>2</sub> is also present in tears and saliva and preferentially targets gram-positive bacteria. SP-A and SP-D are present in pulmonary epithelial lining fluid; they are produced by type II pneumocytes and Clara cells and belong to the pattern recognition receptor family of C-type lectins, which bind to surface carbohydrates on microbial pathogens and promote their phagocytosis by alveolar macrophages.<sup>4</sup>

**The physical barriers presented by epithelial surfaces (skin, upper and lower respiratory tract, gastrointestinal tract, conjunctiva) represent a resilient first-line barrier to most infectious agents**

Epithelial surfaces also contain high concentrations of broad-spectrum, cationic, antimicrobial and antiviral peptides which belong to the histatin, cathelicidin, and defensin families. In the case of antimicrobial activity, these anti-infective peptides/proteins appear to share a common mechanism of action, targeting the outer membrane, resulting in membrane disruption and dysfunction. Histatins 1, 3 and 5, which consist of 38, 34 and 22 amino acids respectively, are present in the saliva of humans, being secreted by the parotid and sub-mandibular salivary glands.<sup>5</sup> They are

preferentially active against fungi, with more limited antibacterial activity. Cathelicidins are a diverse family of anti-infective peptides which target bacteria, fungi, yeasts and viruses.<sup>6,7</sup> The human cathelicidin, LL-37, is 37 amino acids in length and is produced constitutively by the epithelia of the respiratory, gastrointestinal and reproductive tracts, as well as by immature neutrophils, monocytes, mast cells, lymphocytes, eccrine and salivary glands.<sup>6</sup>

'Defensin' is the collective term for a large family of anti-infective peptides which, like cathelicidins, possess broad-spectrum anti-infective properties encompassing gram-positive and gram-negative bacteria, fungi and yeasts, as well as many enveloped and non-enveloped viruses.<sup>3,7</sup> The defensins consist of two major sub-families known as the alpha-defensins and beta-defensins. These differ according to structure, amino acid composition and cellular origin, while the individual members of each sub-family vary with respect to size (18 - 45 amino acids) and target pathogens. The alpha-defensin family consists of 6 members, 4 of which are found in the primary granules of neutrophils (accounting for 30% of total granule protein), and 2 in the Paneth cells of the crypts of the small intestine.<sup>7</sup> These are known as human neutrophil peptides 1-4 (HNP 1-4) and human defensins 5 and 6 (HD5 and 6). There are 4 human beta-defensins (HBD-1-4) and these are essentially synthesised in the epithelial compartment, with production being both constitutive and inducible (induction of HBD-2 and HBD-3 occurs during infectious states).<sup>7</sup>

Subversion of the aforementioned mechanical, expulsive and antimicrobial protein/peptide-mediated epithelial innate defences necessitates the mobilisation of circulating neutrophils and production of types I and III antiviral interferons by the pattern recognition receptors of the innate immune system.

### **Pattern recognition receptors (PRRs)**

These receptors are found on/in the cells of both the innate and adaptive immune systems, and are also expressed by epithelial

cells and endothelial cells. As mentioned above in the case of C-type lectins, they recognise and bind to molecular structures which are common to pathogenic microorganisms and viruses, but which are not found on human cells. Other well-characterised families of PRRs in humans are the Toll-like receptors (TLRs), of which there are at least 11 family members, the nucleotide oligomerisation domain-like receptors (NLRs) consisting of 22 family members, and the abundant cytosolic microbial and viral DNA sensors.<sup>9</sup>

**The pro-inflammatory cytokines/chemokines and histamine and the vasoactive peptide, bradykinin, initiate a local inflammatory response characterised by the early influx of neutrophils.**

### **Toll-like receptors (TLRs)**

TLRs 1,2,4,5,6 and 11 are located on the plasma membrane of cells of the innate immune system and epithelial cells and recognise a range of pathogen-associated molecular patterns found on the cell-wall of bacteria, including lipopeptides (TLRs1 and 6), lipoteichoic acids and lipoproteins (TLR2), lipopolysaccharides (TLR4), and bacterial flagellin (TLR5), while TLR11 interacts with less well-defined ligands on uropathogenic bacteria;<sup>3</sup> the remaining TLRs are located cytoplasmically in endosomes where they interact with viral double-stranded (TLR3) and single-stranded RNA (TLRs7 and 8), and with bacterial and viral DNA via interactions with so-called unmethylated CpG sites (TLR9), which are rarely encountered on the human genome.<sup>9</sup> TLR10 has no known function in humans.

The interaction of TLRs with their respective microbial or viral ligands initiates a cascade of intracellular signalling events which result

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in the activation of cytosolic transcription factors, most commonly nuclear factor kappa B (NFκB) and/or the interferon regulatory transcription factors 3 and 7 (IRF 3/7).<sup>8-10</sup> The activated transcription factors then translocate to the cell nucleus where they induce the expression of genes encoding pro-inflammatory cytokines and chemokines, beta-defensins, and type I antiviral interferons (IFN-α and IFN-β), as well as the more recently described type III interferon (IFN-λ).<sup>11,12</sup> The cytokines interleukin (IL)-1β, IL-6, and tumour necrosis factor (TNF) cooperate with the chemokines IL-8 (CXCL8) and monocyte chemoattractant protein (MCP-1, CCL2) to promote the transendothelial migration and chemotaxis of neutrophils and monocytes, facilitated by TLR-mediated release of histamine from tissue mast cells.

### Nucleotide oligomerisation domain-like receptors (NODS/NLRs)

This family of 22 intracellular PRRs recognises highly conserved structures on bacterial pathogens. NOD1 recognises peptidoglycans mainly produced by gram-negative bacteria, while NOD2 generally recognises those produced by all bacteria. In both cases, activation of NFκB results in pro-inflammatory gene expression.<sup>8</sup> Other members of this family, such as the NLRs, NLRP1, 3 and 4, form a proteolytic complex known as the inflammasome on interaction with their microbial ligands. This, in turn, mediates the conversion of newly synthesised pro-IL-1β to the active cytokine.<sup>8</sup>

### Cytoplasmic microbial and viral nucleic acid sensors

A number of different cytosolic sensors of pathogen-derived nucleic acid have been described.<sup>8-10</sup> As is the case with NLRs, the interaction of these sensors with microbial DNA, or viral DNA or RNA, upregulates host defences by the following mechanisms:

- NFκB-mediated activation of pro-inflammatory genes and beta-defensins
- IRF 3/7- and NFκB-mediated activation of the IFN-α, IFN-β and IFN-λ genes
- formation of inflammasomes.

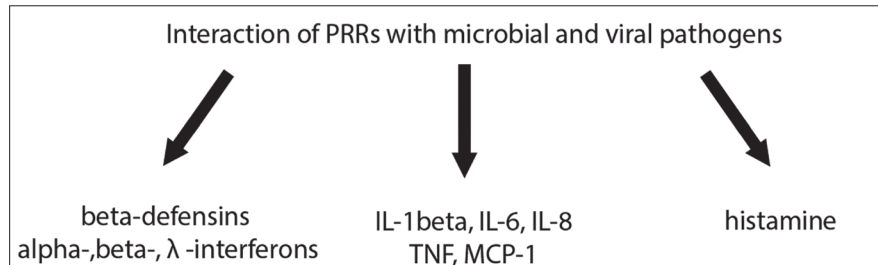


Fig. 1. Release of anti-infective peptides/proteins, pro-inflammatory chemokines/cytokines, and histamine following interaction of PRRs on cells of the innate immune system and epithelial cells with conserved molecular structures on microbial and viral pathogens.

These events are summarised in Fig. 1

### The inflammatory response

The pro-inflammatory cytokines/chemokines and histamine and the vasoactive peptide, bradykinin, initiate a local inflammatory response characterised by the early influx of neutrophils. The events involved are as follows:

- complement activation
- upregulation of expression of endothelial adhesion molecules
- adherence of neutrophils (and monocytes) to vascular endothelium
- transendothelial migration
- chemotaxis to sites of infection.

### Complement activation

Activation of the complement cascade during innate immunity is preceded by (i) synthesis of the acute phase reactant, mannan-binding lectin (MBL) by IL-6-activated hepatocytes; and (ii) histamine/bradykinin-mediated contraction of vascular endothelial cells via interaction with types H<sub>1</sub> and B<sub>2</sub> receptors respectively, widening the gaps between the cells. This, in turn, promotes increased vascular permeability, with consequent leakage of blood proteins, including those of the complement system and MBL. Binding of complement component C3b and MBL to the surface of invasive pathogens then initiates activation of the alternative and MBL complement pathways respectively. Both of these activators (C3b and MBL) possess the intrinsic ability to recognise and bind to the surface of microbial pathogens.<sup>14</sup> Although the pathogen recognition mechanisms differ, the primary consequence of activation of both pathways is identical, namely the

generation of a series of complement-derived pro-inflammatory polypeptides.

These are:

- C3a and C5a (promote neutrophil/monocyte chemotaxis and mast cell degranulation)
- C3e (promotes the release of neutrophils from the bone marrow)
- C3b and C3bi (possess opsonic activity promoting the phagocytosis of pathogens).

In addition to these, the late-acting components of the complement system, which are common to both pathways, polymerise to form a complex, C5b,6,7,8,9 which perforates the cell wall of the pathogen.

### Activation of vascular endothelium

Localised egress of neutrophils from the circulation requires up-regulation of the expression of adhesion molecules on, and presentation of, chemo-attractants by vascular endothelium. Altered expression of endothelial adhesion molecules is achieved by two mechanisms, one involving histamine (as well as thrombin, and reactive oxygen species), and the other the cytokines, especially IL-1β and TNF. Interaction of histamine with H<sub>1</sub> receptors on vascular endothelial cells results in the rapid mobilisation of Weibel-Palade granules to the outer membrane.<sup>13</sup> These granules are a reservoir of the endothelial adhesion molecule, P-selectin. The cytokines, IL-1β and TNF, on the other hand, induce the synthesis and upregulated expression of the endothelial adhesion molecules E-selectin, intercellular adhesion molecules

(ICAMs)-1 and -2, and vascular adhesion molecule (VCAM)-1.<sup>14,15</sup>

**Elevated body temperatures associated with acute infection are activated by innate host defence mechanisms, specifically by the cytokines mentioned earlier, IL-1beta, IL-6 and TNF, which also function as endogenous pyrogens.**

The initial binding of circulating neutrophils to activated endothelium is relatively weak, involving interactions of P- and E-selectins with their counter-receptors on neutrophils, these being L-selectin and several membrane glycoproteins with terminal sialyl Lewis X oligosaccharide groups. This loose interaction, known as 'tethering and rolling', facilitates the interaction of ICAM-1 and ICAM-2 with their counter-receptors on the neutrophil membrane, known as beta<sub>2</sub>-integrins.<sup>14,15</sup> At this stage, the fate of the adherent neutrophils is dependent on the presence of chemo-attractants presented by endothelial surface proteoglycans. In the absence of chemo-attractants, the neutrophils simply detach, while in their presence, recognition by neutrophil counter-receptors for chemo-attractants initiates the activation of beta<sub>2</sub>-integrins, resulting in firm binding. Adherent neutrophils then undergo the process of transendothelial migration, exiting the circulation by squeezing through the spaces between endothelial cells, a process involving (i) endothelial junctional adhesion molecules and (ii) neutrophil membrane proteases which dissolve the basement membrane.

These cells then reach the site of the invasive pathogen by mechanisms involving gradient

**Table 2. Neutrophil-derived anti-infective agents**

Preformed granule proteins*	Newly synthesised reactive oxygen and nitrogen species
Bactericidal proteins	Superoxide
Defensins (HNPI-4)	Hydrogen peroxide
LL-37	Hypohalous acids
Myeloperoxidase	Hydroxyl radical
Proteinases (cathepsins, elastase, proteinase-3)	Singlet molecular oxygen
Secretory phospholipase A <sub>2</sub>	Nitric oxide

sensing of chemo-attractants generated at the site of the infection (chemotaxis), and utilisation of adhesion molecules which promote interactions with the extracellular matrix. Neutrophils which arrive at the site of the infection amplify the chemotactic response by releasing the chemoattractants IL-8 and leukotriene B<sub>4</sub>, as well as E-series prostaglandins which promote increased vascular permeability.

The selective egress of leukocyte subpopulations from the circulation is determined by (i) the type of chemoattractant presented by vascular endothelial cells; and (ii) differential utilisation of adhesion molecules. For example, monocytes, unlike neutrophils, utilise a beta<sub>1</sub>-integrin, which interacts with VCAM-1.<sup>14,15</sup>

### Phagocytosis and elimination of pathogens

On reaching the site of infection, neutrophils as well as monocytes/macrophages, utilise a range of membrane receptors to promote attachment to, and ingestion of, target pathogens. These include:

- TLRs 1, 2, 4, 5, 6, and 11
- the complement receptors CR1, CR3, and CR4, which target the split complement products C3b and iC3b bound to the pathogen
- the acute phase reactant, C-reactive protein, which binds to the C-polysaccharide of many bacteria, promoting phagocytosis
- several members of the C-type lectin family.

This latter group, the opsono-phagocytic family of C-type lectins, functions pre-

dominantly as acute phase reactants. They are calcium-dependent PRR proteins that recognise and bind to microbial carbohydrates and promote phagocytosis via complementary receptors on neutrophils and monocytes/macrophages. Important examples include MBL, SP-A and SP-D, class A scavenger receptors such as MARCO (macrophage receptor with a collagenous structure), and dectin-1 and dectin-2.<sup>4,16</sup> Adherence of opsonised pathogens to neutrophils and monocytes/macrophages initiates the process of internalisation, resulting in the entrapment of the pathogen in a membrane-bound, cytoplasmic structure known as the phagosome. Here the pathogen is bombarded by an array of two main types of toxic agents: (i) preformed anti-infective granule proteins; and (ii) newly synthesised reactive oxygen and reactive nitrogen species generated by the enzymes NADPH oxidase (acting in concert with the primary granule enzyme myeloperoxidase) and nitric oxide synthase, respectively. Examples of these are shown in Table 2.

In addition, dying neutrophils, through a process known as NETosis, release neutrophil extracellular traps (NETs). These are webs of chromatin (of nuclear origin, consisting of nucleic acids and histones), heavily impregnated with granule-derived antimicrobial proteins, which entrap and neutralise microbial pathogens.<sup>17</sup>

### Natural killer (NK) and γ: δ T cells

There are several types of NK cells and γ: δ T cells which recognise their targets without prior sensitisation. NK cells are large granular lymphocytes which

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mediate the death of tumour cells and virus-infected cells via targeted release of cytotoxins from their cytoplasmic granules and/or induction of apoptosis (programmed cell death). These cells account for 10 - 15% of blood lymphocytes and may increase significantly in number during viral infections. Although recognised as a cellular element of the innate, as opposed to the adaptive, immune system, the pathogen-associated targets of  $\gamma$ :  $\delta$  T cells, which make up 1 - 5% of blood lymphocytes, remain to be conclusively established.

### Fever

Elevated body temperatures associated with acute infection are activated by innate host defence mechanisms, specifically by the cytokines mentioned earlier, IL-1 $\beta$ , IL-6 and TNF, which also function as endogenous pyrogens. Notwithstanding inhibitory effects on the proliferation/replication of fastidious pathogens, increasing body temperature is associated with progressive increases in the generation of antimicrobial reactive oxygen and nitrogen species by

neutrophils, as well as augmentation of the functions of NK cells.<sup>18,19</sup>

### Interactions between innate and adaptive immunity

Although innate immune mechanisms *per se* may be adequate to prevent or eradicate infection, they are also critically involved in programming and recruiting adaptive immune mechanisms. This, in turn, results in mobilisation of both innate and adaptive host defences on a subsequent encounter with the pathogen, maximising protective efficacy. Some important examples include:

- antigen delivery to regional lymph nodes and presentation to helper T lymphocytes by dendritic cells
- recruitment of effector T lymphocytes to sites of infection by chemokines released by cells of the innate immune system and epithelial cells
- cells of the innate immune system can orchestrate the differentiation of T lymphocytes along the Th1, Th2, or Th17 pathways
- complement activation is most effective when the alternative and MBL pathways

are augmented by the antibody (IgG and IgM)-dependent classic pathway of complement activation.

### Conclusions

Notwithstanding its barrier and expulsive functions, as well as the anti-infective activities of chemicals and glycoproteins/proteins present in epithelial lining fluid, the innate immune system utilises PRRs to mobilise and recruit inflammatory cells to sites of infection. The critical involvement of innate immunity in host defence is underscored by the recurrent and chronic life-threatening infections associated with:

- cystic fibrosis and primary ciliary dyskinesia due to dysfunction of the mucociliary escalator
- acquired and primary immunodeficiency disorders associated with abnormalities of neutrophil production or function
- deficiencies of complement components
- defective signalling through the IFN- $\beta$  receptor.

References available at [www.cmej.org.za](http://www.cmej.org.za)

### IN A NUTSHELL

- Innate immunity is the first-line of host defence.
- It encompasses mechanical barriers, the mucociliary escalator, and anti-infective chemicals and peptides/proteins.
- If subverted, pattern recognition receptors confer the next level of protection.
- This is characterised by the release of anti-infective interferons and defensins, as well as pro-inflammatory cytokines.
- The cytokines interact with the complement system to trigger neutrophil influx.
- Neutrophils may eradicate or restrict the spread of microbial pathogens.
- Phagocytosis of pathogens is mediated by pattern recognition and complement receptors.
- Neutrophil antimicrobial activity is mediated by reactive oxygen species and granule proteins.
- Innate immune mechanisms are enhanced by elevated body temperature.
- Dying neutrophils contribute to innate immunity by releasing extracellular nets.