Pulmonary defences to acute respiratory infection

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Of all sites in the body, the lung is perhaps challenged by the greatest onslaught of microbial pathogens, many of which would cause lethal infections if unopposed. The immune response to respiratory infection must, therefore, be rapid and efficient. However, the respiratory tract is a fragile tissue with architecture that is finely designed for gas exchange, so that the price of excessive or inappropriate inflammatory responses may itself be very high. The first line of defence comes from barriers such as mucus and cilia, followed by a battery of mediators that constitute the innate response. These include lactoferrin, lysozyme, collectins and defensins. Activation of these molecules can lead directly to lysis of pathogens, or to destruction through opsonisation or the recruitment of inflammatory cells. The adaptive immune response includes the production of neutralising antibodies and the responses of T lymphocytes. Different populations of T lymphocytes may dramatically alter the balance between clearance of the pathogen and induction of tissue damage depending on the cytokines they secrete.

Most pathogens gain entry across mucosal surfaces and the lung, representing the largest epithelial surface in the body, constitutes the major portal for entry of many micro-organisms. During respiration, the airways are exposed to continual challenge by an enormous load of airborne micro-organisms and must, therefore, employ robust mechanisms – in the first instance to offer physical barriers to entry, and failing this, to mount innate or specific immune responses. The cystic fibrosis (CF) lung and the AIDS lung offer examples of the respiratory infections that can thrive if elements of the innate response in the first case, or the specific immune response in the second, become impaired.

An important facet of the specific immune response to infection in the lung is the necessity to mount an appropriate and regulated immune response so as to clear infection rapidly, while not exposing the tissue to chronic inflammation which is itself pathogenic. In recent years, considerable attention has been paid to the role in protection and pathogenesis of the specific cytokines made by activated T lymphocytes. The
balance between cytokines that may provoke, for example, bronchial infiltrates that are primarily either neutrophilic or eosinophilic, will have a major impact on disease. Immunity in the lung faces the particular problem that, at the same time as requiring the capacity for rapid and potent clearance of infection, any overshoot in cytokine responses or inappropriate cellular response may cause inflammatory changes that alter the function and architecture of the airways. Many chronic conditions of the lung including bronchiectasis and sarcoidosis may represent diseases of disregulated immunity to local infection.

The aim of this article is to introduce current concepts from research into innate and specific immunity to infection in the lung. It will be seen that the lung offers physical barriers to ingress of micro-organisms, then a multilayered system of evolutionarily primitive, cellular responses to the recognition of structurally-conserved features of the pathogens and, finally, a highly regulated, specific, immune response involving both antibodies and subpopulations of T lymphocytes to the microbial antigens. A recurrent theme is the balance between fighting infection and colateral tissue damage and consequent impairment of respiratory function. In view of the nature of the mechanistic questions of the functions of specific cell types and molecules, examples are drawn from experimental mouse models of infection as well as from clinical evidence. Many of the experiments use either transgenic mice (that is, mice expressing or over-expressing the gene product of interest as a consequence of DNA micro-injection into the oocyte) or knockout mice (that is, mice lacking the gene product of interest due to replacement at the blastocyst stage of the normal gene with a gene copy that has been disrupted to ablate expression).

**Innate immunity**

As micro-organisms enter the respiratory tract, the first obstacles to entry are from mechanical barriers. Mucins of the mucociliary blanket lining the surface of the airways act by trapping micro-organisms that are then cleared by ciliary movement. Particles that pass this barrier are met by a range of soluble mediators, some constitutive and some induced by specific activation, produced by cells of the respiratory tract. The airway surface fluid (ASF) contains several proteins with antimicrobial activity. Many of these mechanisms are highly sensitive to local salt concentrations and are, therefore, believed to be impaired in CF. Another level of innate immunity dysfunction in CF is that the protein that is mutated in this condition, the cystic fibrosis transmembrane conductance regulator (CFTR) is itself a receptor for internalisation and clearance of some micro-organisms, particularly pseudomonas.
Many of the innate responses are considered primitive, not in the sense of being in any way inefficient, but in the sense that they are conserved through evolution. Humans share the use of many related genes and functions with invertebrate species that use these mechanisms in the absence of any adaptive immune response. A feature common to induction of many of the protective factors that contribute to the rapid, innate response is that these mechanisms are often evolved to recognise and respond to conserved structures of micro-organisms. These recognised features may include particular bacterial carbohydrates, lipopolysaccharide (LPS; recognised by Toll-like receptors), or forms of bacterial DNA (unmethylated cytosine-guanosine-rich areas known as CpG sequences) that are not seen in human genes. Analysis of DNA from sputum of CF patients shows that up to 10% of it can be of the CpG, bacterial type. In studies to look at lower airways inflammation following challenge with either mammalian DNA or CpG-rich DNA, it was found that the latter induced a 4-fold increase in cellular infiltrates and a 50-fold increase in the inflammatory cytokine tumour necrosis factor-α (TNF-α)³. Lysozyme is a major constituent of lavage fluid and sputum and represents an important antimicrobial defence, particularly against Gram-positive bacteria. It is made by glandular serous cells, surface epithelial cells and macrophages. In order to examine its role in vivo, transgenic mice have been generated which over-express lysozyme in the lung⁴. Elimination of pulmonary pseudomonas infection as well as group B streptococci was dramatically enhanced leading to a decrease in systemic dissemination, enhanced recruitment of neutrophils and protection from death. Furthermore, clinical study of susceptibility and resistance to acute bronchitis showed a correlation of protection with levels of macrophage-derived lysozyme⁵.

Lactoferrin is another component of the constitutive defences in the airways and is produced by serous cells as well as neutrophils. Like many of the other mucous constituents, it is able to kill and agglutinate bacteria which it recognises on the basis of carbohydrate motifs, as well as stimulating superoxide production by neutrophils⁶,⁷. As with lysozyme, the concentration of lactoferrin is markedly increased in the lower respiratory tract in subjects with chronic bronchitis⁸.

The α- and β-defensins show broad microbicidal activity against Gram-negative and Gram-positive bacteria, mycobacteria, fungi and some viruses. They are not specific to the lung, being found at other mucosal surfaces including the gut and reproductive tract. They act by inducing permeabilisation and are up-regulated in the lung in response to the inflammatory cytokine, interleukin-1 (IL-1)⁹. Binding of defensins to complement components also leads to triggering the alternative complement cascade. The function of the defensins is highly salt concentration dependent and likely to be impaired in CF⁷,¹⁰. One of the
best characterised members of the β-defensin family is tracheal antimicrobial peptide (TAP) which is highly expressed in the ciliated airway epithelium and is up-regulated in response to bacterial LPS.

The collectins are a large family of proteins which have the ability to recognise and bind to carbohydrates on the surface of pathogens including both bacteria and viruses. This triggers the recruitment of other cells and defensive mechanisms, including activation of the alternate complement cascade. The collectins can also have direct effects on activation of immune cells including macrophages and lymphocytes. Key members of the collectin family include surfactant proteins A and D (SP-A and SP-D) and mannan-binding lectin (MBL). Again, much of our knowledge comes from knockout mice, lacking the genes for these products. SP-A deficient mice are highly susceptible to group B streptococcal pneumonia and sepsis after intratracheal infection. On infection with pseudomonas, the knockouts show a relative inability to destroy bacteria and a decrease in phagocytosis by alveolar macrophages.

Immunoglobulin A (IgA) is the major immunoglobulin found in the healthy respiratory tract. It is believed to be the most important immunoglobulin for defence at this site, a view borne out by the susceptibility to chronic bronchial infections in patients with IgA immunodeficiencies. Like other immunoglobulins, it must be made and secreted by B lymphocytes. However, unlike the other immunoglobulins found in the lung, particularly IgG and IgE, it is not part of the T lymphocyte-dependent immune response. CD4 T-cell knockout mice demonstrate normal IgA responses. It is believed that IgA-secreting B-cells may be stimulated locally in the lung through the release of stimulatory cytokines from epithelial cells. The IgA response is an important component of rapid, local lung immune responses to infection by viruses including influenza and respiratory syncytial virus (RSV).

Another rapid response to local viral infection comes from the type I interferons (IFNs), IFN-α and IFN-β. This is part of the direct, autonomous response of cells in the respiratory tract, including epithelial cells and macrophages, to viral infection. As a consequence of type I interferon release, a number of factors are released which have the ability to interfere with viral replication. Thus, knockout mice lacking the receptor for these interferons show enhanced viral replication in the lung, although, rather than causing increased lethality, protection is maintained through an augmented neutralising antibody response. Furthermore, resolution of infection in these circumstances can be associated with an altered inflammatory response, with the appearance of granulocytic pulmonary inflammatory cells. As with many of the other knockout mouse studies, this demonstrates that, often in lung immunity there are multiple layers of defence, this redundancy helping to ensure that no single deficiency necessarily allows lethal infection. However, changing the fine balance of immune mechanisms used to
fight a particular pathogen may lead to an altered inflammatory response and in turn to inflammatory tissue damage.

Many of the pathways described above lead to the release of mediators that have the effect of increasing neutrophil migration to the lung. Within a few hours of experimental infection or LPS injection, there is massive neutrophil recruitment until these cells constitute 60–80% of bronchial alveolar lavage (BAL) cells. This can occur through the stimulation of factors including IL-8, complement activation or the release of chemokines. Activated neutrophils have an enormous capacity to phagocytose and neutralise bacteria, and can also secrete various factors including defensins, TNF-α, interleukin-1β (IL-1β) and IL-6. For example, experimental infection with *Haemophilus influenzae* causes respiratory epithelial cells to both release IL-8 and up-regulate expression of a molecule called intercellular adhesion molecule-1 (ICAM-1). The IL-8 leads to neutrophil recruitment and the enhanced ICAM-1 to increased neutrophil adherence, both of these contributing to clearance of the infection.

Viruses attempting to infect cells in the lung face potential attack from one more cell-type of the rapid, innate response, that is, natural killer (NK) cells. NK cells derive from the same haematopoietic lineage as T lymphocytes, but, unlike those cells, do not have to mature in the thymus and do not express re-arranged antigen receptors. NK cells use what is now appreciated to be a large number of families of cellular receptors to survey the body, looking for cells that, for reasons of viral infection or transformation, have altered expression of human leukocyte antigen (HLA) class I tissue antigens. If the NK cells fail to receive a cellular signal that normal HLA class I has been recognised, they enter a programme of activation leading to lysis of the infected cell and release of interferon-γ (IFN-γ). This may in turn lead to recruitment of other cells. For example, in experimental RSV infection, there is an extremely rapid antiviral NK cell IFN-γ response that precedes and leads to recruitment of virus-specific, cytotoxic T lymphocytes. Local release of IL-12 is an important early event leading to stimulation of NK cells for such rapid anti-viral responses in the lung.

**Specific immunity**

As at other sites in the body, specific immunity to infection in the lung depends on the specific recognition of foreign, microbial antigens by the receptors of B lymphocytes and T lymphocytes. Following prior stimulation of B lymphocytes, either by infection or vaccination, some level of circulating, specific IgG antibody will remain. The antibodies against a given pathogen may be directed against several different binding
sites (epitopes). Some of these antibodies have the ability to neutralise infections through interference with a vital, structural component of the microbe. Other antibodies may act to clear infections through opsonisation for phagocytosis and through activation of the complement system. After initial exposure, a state of B-cell memory is established, leading to the ability to mount a very rapid IgG antibody response on any subsequent re-exposure. Furthermore, the B lymphocyte-derived IgG response is unique in the immune response in showing affinity maturation. This is the ability to introduce small numbers of random mutations into the genes for the antibody, somatically mutating the sequence so that receptors with better affinity for the epitope are selected at each generation of cell division.

The antibody response to infection mounted by B lymphocytes acts in parallel with the T lymphocyte response. This is the other major population of lymphocytes: they carry the surface markers CD3 along with either CD4 or CD8, mature in the thymus and express an antigen receptor that must recognise peptide fragments of microbial antigen presented by host HLA molecules. The major part of T lymphocyte effector function is through the release of a wide range of soluble mediators called cytokines.

In order to consider the contribution of T lymphocytes to immunity and tissue pathology in the lung, it is worth outlining the different types of T lymphocytes involved. During early attempts to identify the antigen receptor used by T lymphocytes, two types of receptor were identified, one of which became known as the $\alpha\beta$ receptor, the other the $\gamma\delta$ receptor. The cells expressing the $\alpha\beta$ T cell receptor are the normal CD4 and CD8 populations with the properties of mounting cytolytic responses to infected cells, making cytokines and stimulating B cells. They do this in response to recognition of self HLA molecules bound to microbial peptides. The T cells expressing the $\gamma\delta$ receptor are particularly associated with defence at epithelial surfaces, have a different mode of recognising foreign antigens, but are also involved in lung immunity.

The $\alpha\beta$ T lymphocytes expressing CD4 have been termed T helper cells. This is because their effector function primarily involves the release of cytokines that have the effect of stimulating or activating some other cell type. This can include the action of IL-4 to stimulate B cells and attract eosinophils, IL-2 which drives other T lymphocytes, including CD8 cells to divide, and IFN-$\gamma$ which both causes other T lymphocytes to differentiate at the same time as activating macrophages and dendritic cells. In some systems, CD4 cells can also be cytolytic through the release of TNF-$\alpha$. $\alpha\beta$ T lymphocytes expressing CD8 are termed cytotoxic cells. On recognition of microbial peptides presented by HLA class I molecules, their primary function is to lyse the infected cell, particularly by release of granules of perforin.
Immune responses to acute respiratory infection

Fig. 1 Activation of Th1 and Th2 CD4 T-cell responses in the lung.

PROTECTIVE RESPONSES:
- Viral and mycobacterial immunity
- Through IFNγ, macrophage activation, cytotoxic T cell activation
- Bacterial clearance through macrophage and neutrophil activation, stimulation of neutralising/opsonising antibody production

PATHOGENIC RESPONSES:
- "T cell and antibody immunity to viral and bacterial infection
- "Excessive activation of local eosinophils and IgE production
- "Asthma, aspergillosis

Stimulation of B lymphocytes to secrete specific IgG1 and IgE
Activation of cytotoxic T lymphocytes (CD8 T cells)
Attraction of eosinophils and basophils

Stimulation of B lymphocytes to secrete specific IgG2a
Activation of neutrophils and monocytes

***Th1 or Th2 choice can depend on nature of organism, type of antigen presenting cell, cytokine microenvironment, expression of costimulatory molecules, affinity of antigenic peptide/MHC for T cell receptor***

**MHC**
- Antigenic peptide
- T cell receptor (specific recognition of peptide from foreign organism)

**Th0 CELL**

**Th1 CELL**
- Interferon-γ
- TNFα

**Th2 CELL**
- Interleukin-4
- Interleukin-5
- Interleukin-10

**ANTIGEN PRESENTING CELL (DENDRITIC CELL, MACROPHAGE, B CELL)**
CD4 and CD8 lymphocyte can be further subdivided on the basis of the array of cytokines they secrete. CD4 cells have been subdivided into (T helper-1 and 2) Th1 and Th2 subpopulations (Fig. 1). Th1 cells are primarily driven to differentiate by the presence of the cytokines IL-12 and IL-18, and, in response to antigen, can make IFN-γ as well as IL-2 and TNF-α. Thus, these cells have classically been regarded as the type of cell that would arrive at a delayed hypersensitivity response to local viral or bacterial infection, stimulating local macrophage activation, neutrophil recruitment and specific cytotoxic T cell responses. In the context of respiratory immunology, sarcoidosis represents an overtly Th1 environment in which the majority of CD4 T cells express receptors for IL-12 and make Th1 cytokines. Th2 cells are primarily driven to differentiate by the presence of the cytokine, IL-4. On encountering antigen, they respond by making more IL-4, IL-5 and sometimes IL-10. This profile of cytokine responses is important for driving B lymphocytes to make antibody responses and, in particular, to switch antibody production to making IgG1a and IgE. These cytokines can also lead to recruitment and activation of basophils and eosinophils. Thus, in the respiratory context, a prototypic Th2 response would be that seen in atopic asthma or in aspergillosis. The examples of sarcoidosis on the one hand and aspergillosis on the other serve to re-iterate the point that the inflammatory response involved in clearing an infection may itself contribute to lung damage. The distinction of CD4 T helper cells into Th1 and Th2 cells was subsequently extended to the subdivision of cytolytic CD8 cells into Tc1 and Tc2. This distinction follows the same split as for T helper cells, Tc1 being primarily characterised by release of IFN-γ and Tc2 primarily by release of IL-4. In the inflammatory responses in other tissues, for example analysis of the cellular autoimmune response in type I diabetes, it is sometimes argued that Th1 responses are ‘bad’, pro-inflammatory and pathogenic, whilst Th2 responses are ‘good’, anti-inflammatory and protective. It is immediately evident that no such distinction can be made in the lung, in which either kind of response may serve a vital role in clearing infection while at the same time having the ability to cause inflammatory tissue damage if not adequately controlled.

Using mouse models of infection and again incorporating transgenic and knockout strains, efforts have been made to characterise the contributions of different T-cell populations to protection and tissue damage. Early studies to look at the protection of mice from influenza infection by adoptive transfer of cultured T cells showed initially that protection was particularly related to the transfer of IFN-γ secreting CD8 cells and subsequently that CD4 cells could also protect. While Th1 CD4 cells could protect against lethal challenge, transfer of Th2 cells exacerbated disease. The double-edged sword of these protective T-cell responses was
demonstrated in a study where transgenic mice were made to express influenza antigen on alveolar epithelial cells. In the absence of any viral infection, transfer of specific CD8 T-cells was sufficient to trigger an inflammatory cascade producing the appearance of an interstitial pneumonia. In recent years, it has become possible to enumerate numbers of systemic antiviral T-cells using fluorescent peptide/MHC tetramers as a probe. From these studies it is apparent that there is incredibly robust and long-lasting CD8 immunity following viral infection, with cytotoxic precursor cells detectable at more than 20-fold increased levels for life. This memory ensures that on re-challenge with virus, immune individuals can clear virus considerably faster than immunologically naïve individuals. The ability of CD8 cells to clear a pulmonary viral infection is not simply related either to the number of cells or to their cytolytic capacity as Tc1 cells are able to isolate and clear influenza infections far more rapidly than equally cytolytic Tc2 cells. This is likely to reflect the fact that these subpopulations are able to migrate to the lung with different efficiencies and also have different capacities to attract other antiviral populations. When considering the differing contributions of these T lymphocyte subpopulations to protection and inflammation in the context of BAL measurements, it is worth noting that comparative analysis of T lymphocytes from parenchyma, airways or mediastinal lymph nodes shows distinct populations.

In view of the many experimental systems in which there is a distinct impact from T lymphocytes making particular Th1 or Th2 cytokines, there have been many attempts to investigate the effect of specific cytokines on clearance of infection. In mice expressing transgenic IL-4 in the lung, there is delayed clearance of virus, although this in turn leads to an enhanced neutralising antibody response. This re-inforces the general principle that there are many parallel mechanisms for clearing infection from the lung, although these may be associated with different inflammatory consequences. A case in point is experimental infection with RSV, in which immunisation-induced Th2 eosinophilia can be reversed by treatment with the Th1-skewing cytokine, IL-12. However, despite reversing the eosinophilia, the clinical outcome is relatively unchanged. As would be predicted from the T lymphocyte transfer studies, many studies show impaired clearance of infection in mice lacking IFN-γ or IFN-γ receptors. For example, IFN-γ knockout mice show very rapid progression of Mycobacterium tuberculosis infection and there is impaired ability to clear influenza infections. Mice lacking IL-12, which can be considered the cytokine upstream of IFN-γ in the Th1 pathway, also show greatly enhanced susceptibility to mycobacterial infection.

The critical importance of understanding these sometimes subtle differences in protective cytokine responses is demonstrated by experiences
with RSV vaccine design. RSV vaccine trials in the 1960s led to enhanced morbidity and mortality on exposure to live virus in those vaccinated compared with controls. This was associated with peribronchiolar infiltration and excess eosinophils in the lungs and blood. Mortality is thus likely to have been associated with priming for a pathogenic, excessive Th2 response. It has recently been possible to dissect out the viral proteins responsible for damaging eosinophilia and create vaccines for use in mouse models which are capable of inducing protection without the associated pathological consequences.

The role of $\gamma\delta$ T cells in protection from respiratory pathogens is less well characterised than TCR $\alpha\beta$ CD4 and CD8 cells. Nevertheless, work has been done to compare susceptibility to infection in knockout mice lacking TCR $\alpha\beta$ cells compared with those lacking TCR $\gamma\delta$ cells. The latter knockouts are extremely susceptible to mycobacterial and nocardia infections. Surprisingly, mice lacking TCR $\gamma\delta$ cells are substantially more susceptible to lethal bacterial pneumonia following infection with Klebsiella.

**Key points for clinical practice**

- The immune response to microbial challenge in the lung is multilayered with defences ranging from physical barriers to innate responses and the adaptive response mediated by antigen specific T and B lymphocytes.
- The benefit of clearance of the infection must be balanced against the cost of tissue pathology and potential impairment of lung function.
- In future clinical practice, it will become increasingly important to analyse the immune parameters of responses in the lung to develop a better understanding of the origins and control of chronic inflammatory disease.
- This should have impact on the development of new therapeutic strategies and the design of rational, safe vaccines.

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**References**

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15 Salvi S, Holgate ST. Could the airway epithelium play an important role in mucosal immunoglobulin A production? Clin Exp Allergy 1999; 29: 1597–605
22 Kurup VP, Choi HY, Murali PS et al. Immune responses to Aspergillus antigen in IL-4–/– mice and the effect of eosinophil ablation. Allergy 1999; 54: 420–7
30 Hussell T, Khan U, Openshaw P. IL-12 treatment attenuates T helper cell type 2 and B cell responses but does not improve vaccine-enhanced lung illness. *J Immunol* 1997; 159: 328–34


34 Kapikian AZ, Mitchell RH, Chanock RM *et al*. An epidemiologic study of altered reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with inactivated RS virus vaccine. *Am J Epidemiol* 1969; 89: 405–21


