Hidden risks for pneumonia in Malawi

DG Fullerton1, SB Gordon2

1. Department of Respiratory Medicine Hope Hospital, Stott Lane, Salford, M6 8HD, U.K.

ABSTRACT

Domestic smoke exposure and early HIV infection are critical but unseen risk factors for pneumonia. This paper reviews how recent research in Malawi and elsewhere contributes to an understanding of the possible immunological mechanisms underlying these risks.

Introduction

Lower respiratory tract infections account for a major burden of premature death and disability worldwide34. The greatest burden of disease is felt in the developing world where the incidence of acute lower respiratory tract infections (ALRI) is 12-fold higher than in developed countries8,9. Risk factors for ALRI include age (0-11 months), gender (male), lack of breast feeding, HIV infection, malnutrition (both macro and micro-nutrients) and environmental factors such as crowding and indoor air pollution10,11. In Malawi, endemic HIV infection and the common use of smoky fuel for both cooking and lighting are the most common risk factors for respiratory tract infections in adults. This review will focus on how important pulmonary defence mechanisms are compromised by these risk factors.

Normal lung defence

The lung is constantly challenged by inhaled particles and microbial pathogens. The first line of defence consists of mucus-covered ciliated epithelium and the cough reflex35. The next line of defence consists of innate responses that are non-specific host defence responses to foreign particles occurring predominantly at the epithelial surface. Mediators of the innate response include soluble factors and host cells36,37. Finally, humans mount acquired responses to invading pathogens. These are specific humoral (antibody) and opsonophagocytic responses regulated by the cellular immune system. The entire immune response must rapidly remove inflammatory stimuli because the respiratory tract is a fragile tissue with a delicate structure that is designed for gas exchange and excessive inflammation impairs this function38.

Airway clearance, domestic smoke and tobacco smoking in Malawi

Each day approximately 7000 litres of air are drawn through the nose where large particles are deposited. Particulate material larger than 0.5 μm in diameter is deposited in the lining fluid (mucus) of the trachea and bronchi39. Clearance of this debris, particles and secretions is by mucociliary action. The lower airways are ciliated and lined by epithelium that contains goblet cells and submucosal glands. These glands produce enough mucus to cover underlying cilia which constantly sweep the mucus towards the throat where it is swallowed40. In Malawi, the majority of homes use a mixture of fuels for cooking including wood and charcoal, as well as a variety of smoky means of lighting including paraffin tin lamps and candles41. The extent of this smoke exposure can be clearly demonstrated by examining fluid washed from the lungs of patients at bronchoscopy as shown in Figure 1. The effect of this smoke on the ciliary function and mucus production of exposed Malawians has not yet been assessed.

Figure 1

This figure shows bottles of freshly obtained bronchoalveolar lavage taken from 2 different subjects on the same day, and alveolar macrophages obtained from the same samples stained with the fluorescent nucleic acid stain DAPI and viewed under standard transmitted light with simultaneous laser illumination. The left hand sample is dark in color due to carbon particles within the alveolar macrophages that can be seen outlining the cytoplasm under transmitted light (middle panel). The right hand sample is cloudy in color and the macrophage preparation shows cell nuclei staining blue together with blue-staining coagulase-negative staphylococci which were added to the preparation. Supernatant fluid after removal of the cell pellets from both samples was clear.

Mucociliary clearance is substantially impaired by cigarette smoking due to the increased viscosity and volume of mucus produced by smokers as well as the damage to cilia caused by increased levels of proteases in the mucus42. Cigarette smoking is the most important risk factor for pneumonia among immunocompetent adults in the USA43. The health significance
of cigarette smoking in Malawi is not currently known but is an important area for future research as tobacco is a major local cash crop and both commercial and home-rolled cigarette smoking is common. Clinical diagnoses of chronic bronchitis secondary to cigarette smoking are still rare in QECH, Blantyre. Cough can be a voluntary action or a reflex respiratory response to inhaled particulate matter, irritants, high or low temperatures and humidity(9). Most commonly cough is a symptom of infection, but in developed nations it is associated with cigarette smoking. In Malawi, chronic cough is associated with tuberculosis(11). The incidence of cough as a symptom was common among Malawians with HIV and pneumococcal disease, but the duration of cough was not predictive of outcome among hospital admissions(19).

Innate immune factors and lung defence in Malawi

A range of soluble innate immune mediators meets particles that pass through the mucociliary blanket. Some of these factors are produced constantly at low levels and others are induced by specific activation. Lysozyme, first discovered by Flemming in 1921, is a bactericidal protein capable of lysing the carbohydrate polymers that comprise the external membrane of bacteria(20-27). Lactoferrin and secretory leucocyte protease inhibitor (SLPI) are airway defence proteins produced by serous cells as well as neutrophils. Lactoferrin is able to kill and agglutinate bacteria, which it recognizes on the basis of carbohydrate motifs as well as stimulating superoxide production by neutrophils(28). Both lactoferrin and lysozyme are produced in much greater quantities in patients who have chronic bronchitis(29). The α- and β-defensins show broad anti-microbial activity against Gram-negative and Gram-positive bacteria, mycobacteria fungi and some viruses(30). They act by inducing permeabilisation and are up-regulated in the lung in response to the inflammatory cytokine, interleukin-1 (IL-1)(30). The collectins are a family of proteins that bind to carbohydrates on the surface of pathogens. This triggers the alternative complement cascade. Collectins also have direct effects on the activation of immune cells including macrophages and lymphocytes. Key members of this family include – surfactant proteins A and D (SP-A and SP-D) and mannann-binding lectin (MBL)(32,35).

Several of these innate factors have recently been examined in lung fluid from Malawian volunteers to determine their relation with carbon exposure and HIV infection. No significant difference was found in the level of lactoferrin, lysozyme or SLPI in a comparison between HIV negative adults and HIV positive adults with or without a recent history of pneumococcal disease (manuscript submitted). In HIV negative adults, however, there was an increased level of lactoferrin in subjects with high carbon loads in alveolar macrophages. This suggested that exposure to smoke stimulates lactoferrin production in Malawians despite the low incidence of chronic bronchitis. There is scope for much more work on innate pulmonary defence factors in Malawian adults.

Complement proteins are important components of the innate and acquired immune defence and are found within lung secretions(32,35). Patients with complement deficiencies (eg sickle cell patients) experience recurrent infections with capsule organisms including Haemophilus influenzae (30) and Streptococcus pneumoniae (26). There have been no studies to date of complement levels and function in the lungs of Malawian adults. Host cells are also capable of mounting a cellular innate immune response. Several receptors contribute to this response (eg scavenger receptor, mannose receptor) but the recently discovered toll-like receptors are of particular interest(30). These are primitive receptors on host cells that respond to well-conserved structures of microorganisms such as bacterial lipopolysaccharides and carbohydrates or forms of bacterial DNA. The Toll-like receptor (TLR) on the surface of alveolar macrophages (TLR-2) has recently been shown to mediate innate responses to Mycobacterium tuberculosis and Streptococcus pneumoniae in human alveolar macrophages(32,33). The inflammatory response of alveolar macrophages to an intracellular product of S. pneumoniae called pneumolysin, has been shown to be dependent on TLR4. Unpublished data from Blantyre have shown that HIV infected adults have altered baseline expression of TLR-2 and TLR-4 but the significance of these changes in relation to pulmonary defence against S. pneumoniae or M. tuberculosis is still under investigation.

Acquired immunity in the lung

Immunoglobulins are found at all levels of the respiratory tract(34,35). Immunoglobulin in the respiratory tract agglutinates bacteria, activates complement and acts as an opsonin for phagocytosis by resident alveolar macrophages or neutrophils migrating into the parenchyma in response to inflammation(35). IgA is the predominant immunoglobulin of the upper respiratory tract and IgG predominates in the parenchyma. Respiratory epithelium facilitates the secretion of secretory IgA(35). IgG is thought to be produced locally by pulmonary plasma cells that have matured in regional lymph nodes and migrated back to the tissue where antigen was first presented(34). In acute inflammation, the epithelium becomes increasingly permeable and so plasma proteins, including albumin and immunoglobulin, leak in to the respiratory tract(35). Immunoglobulin facilitates phagocytosis by binding to immunoglobulin receptors on macrophages and neutrophils. In patients with IgG deficiency, recurrent respiratory infections are a major problem(36). In studies of HIV patients from the USA, some early work suggested that IgG levels in lung fluid might be low in patients with HIV(37). Several studies have therefore measured the levels of pulmonary immunoglobulins both in serum and the lung fluid of Malawian adults. In Malawian adults with HIV infection, increased levels of IgG, IgM and IgA were found in plasma and lung fluid. The lung fluid levels were not significantly altered by correction (using albumin levels) for the effect of plasma leakage. In addition, pneumococcal polysaccharide specific IgG was particularly increased in the lung following recent pneumococcal disease both in HIV infected and control patients but the increase was significantly greater in HIV infected adults. This increase in total and specific IgG was thought to be due to the increased production of IL-6 in HIV infected adults – this cytokine drives B cell antibody production(38). There is evidence from other parts of the world that genetic variations in the immunoglobulin receptor structure can increase the risk of pneumonia in certain populations(39). This work will begin shortly in Blantyre.

Cellular components of acquired immunity in the lung

Macrophages, dendritic cells and lymphocytes act together in the lung to orchestrate the rapid uptake and removal of foreign particles from the lung. The proportion of alveolar macrophages and the extent of activation of these populations in adult lung fluid is the subject of current study in our laboratory. As previously described, HIV infected adults have increased lymphocytes and B cells in lung fluid, with a vastly increased percentage of CD8 expressing T lymphocytes(40). Alveolar macrophages are the predominant phagocyte of the
alveolar space and are found at a density of approximately one per alveolus. Alveolar macrophages ingest small numbers of particles, bacteria or apoptotic cells and release oxidative agents and proteases on to this material in phagolysosomes. Some bacteria (e.g. *Legionella spp* and *Mycobacteria spp*) can frustrate phagolysosomal processing, either by resistance to the phagosomal contents or by escape into the cytosol. Following digestion in the phagosome, macrophages present antigen to lymphocytes either locally or in the regional lymph node. The precise mechanism by which antigen is transported to the regional lymph nodes is poorly understood in humans, but is likely to involve dendritic cells and other pulmonary macrophages (interstitial macrophages). Overwhelmed macrophages undergo apoptosis and are removed by the mucociliary escalator and cough reflex. Apoptosis is a preferable response compared to necrosis as it reduces the release of inflammatory mediators that might otherwise damage the respiratory epithelium.

HIV infects and affects alveolar macrophages. This leads to altered function of receptor expression, activation, cytokine production, accessory cell function, phagocytosis and apoptosis of the macrophages themselves. Phagocytosis and killing of *Cryptococcus neoformans* and *Pneumocystis carinii* was seen to be impaired in alveolar macrophages from HIV infected subjects, but no impairment of *Staphylococcus aureus* or *S.pneumoniae* was observed. Some differences in alveolar macrophage function may be due to the prevailing cytokine milieu in the alveolus and so these findings may have to be repeated in the light of different lymphocyte populations and cytokine measurements in the lung fluid.

An important aspect of alveolar macrophage function that is often overlooked is anti-inflammatory cytokine production. Alveolar macrophages have a predominantly immunosuppressive influence on the alveolar surface that is altered in favour of phagocytosis and inflammation in the presence of bacteria or viruses. No difference in anti-inflammatory profile was found, however, in the response of Malawian alveolar macrophages to *S.pneumoniae* in vitro (unpublished data).

**Lymphocytes.** In normal individuals, lymphocytes account for 10% of cells harvested from bronchoalveolar lavage (BAL). In addition, lymphoid aggregates and follicles containing mainly B-lymphocytes are found in contact with the visceral surface of the epithelial layer - this is known as bronchus-associated lymphoid tissue (BALT). The lymphocytes harvested from BAL are approximately 99% T-lymphocytes. These cells distinguish self from foreign protein antigen presented by macrophages and B cells to cell surface antigen receptors (TCR). Different sub-populations of T-lymphocytes defined by surface markers have different functions. CD4 expressing cells are helper T cells with a predominant role in regulating antibody responses and activating macrophages. CD8 expressing lymphocytes are cytotoxic cells and produce a cytokine profile that activates macrophages and NK cells to an effective anti-viral response.

**Neutrophils and monocytes.** Make up only 1-2% of lung lavage from healthy individuals but the vast majority of cells in acutely pneumatic lung. A large number of neutrophils are sequestered by the fluid dynamics and small capillaries of the pulmonary circulation and so are effectively on “stand-by” near the airspace. Monocytes are immature macrophages that circulate like lymphocytes and neutrophils and form an important immune reserve, rapidly recruited when necessary. Monocyte emigration follows the neutrophils, starting at 6 hours, peaking at 12-18 hours.

**Cytokines, chemokines and the acute inflammatory response in the lung.** In the face of overwhelming infection, macrophages and lymphocytes generate pro-inflammatory cytokines to recruit polymorphonuclear and monocytes. **Cytokines** are peptides produced by metabolically active cells of the immune system. They have the ability to activate other cells and include proteins such as interleukins, interferons, tumour necrosis factor, and chemokines. Their effects include chemotaxis, degranulation, protein production, cell division and activation, cytoskeletal rearrangement and immunomodulation. Cytokines usually act over short distances and bind to target cell receptors. Recent work in Malawi has shown that alveolar macrophages from HIV infected adults produced decreased IL-1beta in response to *S.pneumoniae* compared to HIV negative adults. In addition, HIV infected adults were found to have increased RANTES (a beta-chemokine) in lung fluid – these pro-inflammatory responses are probably appropriate responses to the challenge posed by the viral infection itself. Carbon loading of alveolar macrophages has not yet been associated with alterations in cytokine production in Malawi but this is under investigation. Acute smoke exposure increased circulating neutrophil counts by a bone marrow effect mediated by increased IL-8 production by alveolar macrophages in a study from Singapore.

**Interaction of carbon exposure and HIV in the lung in Malawi.** Carbon has a pro-inflammatory effect on the lung as evidenced by altered lactoferrin and RANTES levels. HIV is associated with increased immunoglobulin levels and CD8 lymphocytes. It is interesting to speculate that the two influences might interact and indeed there is a difference in the number of alveolar macrophages containing large amounts of carbon between HIV infected and non-infected Malawians (see Figure 2).

**Figure 2**

The mean percentage of macrophages containing carbon is compared between HIV infected and uninfected subjects. There is a higher percentage in non-HIV-infected subjects. Dividing subjects about the median in to high or low carbon, a greater proportion of high carbon containing samples are obtained from HIV negative subjects (Pearson chi=4.2; p=0.039).

The mechanisms to explain why HIV infected Malawians have less carbon in their macrophages could include down-regulation...
to receptor 2-deficient mice are highly susceptible to Streptococcus pneumoniae meningitis because of reduced bacterial clearance and enhanced inflammation. J Infect Dis. 2002;186(6):598-806.


