# Should We Develop an Inhaled Anti-pneumococcal Vaccine for Adults?

Stephen B. Gordon* and Neil French

*Malawi-Liverpool-Wellcome Programme of Clinical Tropical Research, Universities of Malawi and Liverpool (UK), PO Box 30096, Blantyre, Malawi*

**Abstract:** *Streptococcus pneumoniae* is the most important bacterial cause of pneumonia and meningitis among adults. It is also a common cause of bacteraemia among HIV infected adults with rates of disease approaching 100 times normal community incidence figures. Rates of antibiotic resistance are rising among pneumococcal isolates globally and the currently available 23-valent pneumococcal polysaccharide vaccine is ineffective in HIV infected adult populations. The newer conjugate vaccine has been highly effective in children in the developed world. It may also offer some promise in adult risk populations, but it is expensive and has limited serotype coverage. This article reviews the epidemiology of pneumococcal disease, the current state of pneumococcal vaccines, the pathogenesis of pneumococcal disease, the potential advantages of an inhaled vaccine in adults and some of the chemical obstacles to producing such a vaccine.

## INTRODUCTION

*Streptococcus pneumoniae*, the pneumococcus, is a leading cause of morbidity and mortality at all ages throughout the world [1]. Pneumococcal disease primarily affects the young [2], the old (particularly in the presence of cardio-respiratory co-morbidity) [3] and the immunosuppressed, Human Immunodeficiency Virus (HIV) being the most important of these conditions [4,5]. Pneumococcus is the most common cause of community acquired pneumonia at all ages. Incidence rates of invasive pneumococcal disease are 0.07 per 1000 person years in immunocompetent adults but reach 23.4 per 1000 person years( over 300 times increase) in risk groups such as young children, the elderly and adults with AIDS [6]. Recent advances in vaccination have dramatically altered the incidence of pneumococcal disease in children in developed countries, but adult risk groups and children in the developing world remain vulnerable.

## SUSCEPTIBILITY TO PNEUMOCOCCAL DISEASE

*S.pneumoniae* is an asymptomatic upper airway colonising bacterium in 10% of adults and 40% of children in cross-sectional studies. Droplet spread ensures that in close communities, exposure to pneumococci is universal and therefore, the nasopharynx of almost all adults and children are periodically colonised after pneumococci for several months at a time [2,7].

The normal pulmonary and systemic defences described below ensure that mucosal infections (pneumonia, sinusitis and otitis media) or invasive pneumococcal disease (bacteraemia, meningitis and deep infections) are rare in immunocompetent adults. The factors that determine the shift from nasopharyngeal colonisation to mucosal and then invasive disease are summarised below after a discussion of specific environmental factors, pneumococcal virulence factors and host responses that determine the progression to disease in susceptible patient groups [8].

### Environmental Factors

Environmental changes influencing susceptibility to pneumococcal disease include seasonal changes in temperature and humidity which alter conditions on the nasal mucosa [9], recent viral infection causing up-regulation of critical mucosal adherence sites for pneumococci and exposure to domestic and cigarette smoke [10]. The most significant environmental correlate of susceptibility to pneumococcal disease in otherwise immunocompetent adults is cigarette smoking [11], likely due to multiple effects on cough, airway secretions, phagocytic function and non-specific soluble factors of the innate immune defense of the airway. Special conditions of crowding and humidity alter the transmission of disease-causing pneumococci in military barracks [12], mining camps [13] and small crowded homes [14-17].

### Bacterial Factors

Pneumococcal virulence factors include capsular type [18-20], cell wall [21], pneumolysin [22], surface proteins [23-25], autolysin [26], neuraminidase [27], peptide permeases, hydrogen peroxide [28] and IgA protease [29] but many more factors will now be described using molecular investigative techniques to decipher the functional significance of the fully described (www.tigr.org) pneumococcal genome [30,31]. Pneumococcal virulence factors have been well reviewed in a number of recent papers [32-34] and are only reviewed in this paper, in the relevant sections, in terms of how they may present as candidate antigens for a vaccine or alter the likely efficacy of an inhaled vaccine.

### Host Factors

In considering the possible utility of inhaled vaccination as a strategy to prevent pneumococcal disease globally, it is appropriate to consider in particular detail the defects in host

*Address correspondence to this author at the Malawi-Liverpool-Wellcome Programme of Clinical Tropical Research, Universities of Malawi and Liverpool (UK), PO Box 30096, Blantyre, Malawi; Email: sgordon@mlw.medcol.mw and nfrench@mlw.medcol.mw

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response that are responsible for the excess of disease in special risk groups – the young, the old and people with primary or secondary immune deficiency especially that caused by HIV. The additional risk for pneumococcal disease experienced by certain groups with particular genetic constitution may also be important as successful vaccination strategies must overcome the effect of genetic predisposition as well as environmental and bacterial pressures.

### *Young Age*

Young children, particularly those under the age of 2 years, lack the capacity to mount an effective immunological response to carbohydrates and in particular the polysaccharide capsules of capsulate pathogens. B cells expressing CD21 (C3d complement receptors) are an essential part of the splenic architecture necessary for an optimal response to polysaccharide antigen [35,36]. Recent work has shown that a circulating B cell subgroup of splenic origin, termed IgM memory, is deficient in children under the age of 2 as well as in adults post-splenectomy [37]. Nevertheless, not all splenectomised patients have deficient antibody responses to pneumococcal vaccine [38] suggesting that polysaccharide antigen may be effectively presented in other locations such as regional lymph nodes [39].

### *Old Age*

Elderly patients have an increased incidence of pneumonia and invasive pneumococcal disease but no specific immune defect in otherwise fit elderly people has been identified [40]. It is likely, therefore, that much of the excess of pneumococcal disease seen in elderly patients [41] results from co-morbid illness, the immunosuppressive effects of malnutrition and from environmental factors such as residency in old people's homes and cigarette smoking. The increased mortality observed among elderly people with invasive pneumococcal disease [41,42] and pneumonia [3] likely results from the paucity of clinical signs [3] and resulting late severe presentation to hospital [43] as well as from risk factors such as aspiration.

### *Primary and Secondary Immunodeficiency*

Patients with hypogammoglobulinaemia, complement defects, splenic dysfunction, sickle cell disease, nephrotic syndrome and haematological malignancy all exhibit a marked increase in susceptibility to pneumococcal disease [44,45]. Pregnancy, diabetes mellitus, alcoholism, cirrhosis and non-haematological malignancy also increase susceptibility to pneumococcal disease but to a less marked degree. The mechanisms of deficiency are instructive in the these patients, but the number of potential immune defects, the increase in susceptibility to disease and the number of patients involved globally, however, is greatest for patients infected with the HIV [46].

Hypogammaglobulinaemia results in a loss of capsule-specific IgG and specifically $IgG_2$ responses that are critical in opsonophagocytosis of capsulate bacteria [18,47]. Primary hypogammaglobulinaemic patients are treated with prophylactic gamma globulin infusion to prevent pneumococcal disease [48]. Secondary hypogamma-globulinaemia may result as a direct complication of chronic lymphatic leukaemia and decreased responsiveness to polysaccharide antigens has been reported in both bone marrow and solid organ transplants [49-52].

Primary complement deficiencies cause increased susceptibility to pneumococcal disease both due to decreased efficiency of opsonophagocytosis [53,54] and due to a failure of presentation of antigen to B cells using C3d and the CR2 receptor [35,36]. Nephrotic syndrome causes excessive urinary loss of complement proteins and hence secondary complement deficiencies [55].

Patients with sickle cell disease, in whom both immunoglobulin and complement deficiencies are evident, also fail to clear organisms properly from the bloodstream due to defective splenic sequestration of organisms [56,57]. Children under the age of 2 with sickle cell disease have an approximately 150 times increased incidence of invasive pneumococcal disease compared to healthy infants and a 27% mortality associated with these episodes [58].

### *HIV Infection and Susceptibility to Pneumococcal Disease*

HIV infection results in a dramatic decrease in CD4 lymphocytes in the circulation as well as a pulmonary CD8 lymphocytosis and loss of splenic and lymph node architecture [59]. HIV infection results in increased numbers of activated circulating B cells producing non-specific IgG [60] likely driven by an altered cytokine milieu [61-65]. There is evidence of reduced effectiveness in dendritic cell function [66,67] and altered immunoglobulin repetoires [68]. The resulting immune defects have several direct effects on immunity to pneumococcal infection. These include lower levels of circulating capsule and pneumolysin-specific IgG [69], with lower expression of $VH_3$ idiotype IgM and IgG genes in B cells from HIV infected adults [70,71]. Lower levels of specific antibody initially and in response to pneumococcal antigens have been noted in HIV infected children [72] and in adults [73-75]. Anti-pneumococcal antibody function measured *in vitro* is a better predictor of clinical protection than IgG levels alone [76]. Using an opsonophagocytic assay with exogeneous complement and healthy donor PMNs to measure serum opsonic function, Janoff *et al.* showed reduced titres of phagocytosis in 4 out of 6 bacteraemic HIV infected patients compared to HIV negative controls [77]. In the same study, serum pneumococcal killing titres in patients remained less than control subjects even after recuperation. In addition, further work by the same group showed serum pneumococcal killing titre was lower in HIV infected Kenyan women that in seronegative subjects and proportional to the measured capsule-specific antibody [78]. Nevertheless, there is evidence that response to recall antigen is preserved in HIV infected patients [79].

Studies of humoral defense against pneumococci using broncho-alveolar lavage fluid in several laboratories including our own have not shown evidence of defective IgG production in HIV infected adults [80] but altered IgA has been demonstrated [81]. These responses have been explained by the relatively conserved mucosal plasma cell repetoire in HIV infection [82]. Upper airway washings contained similar levels of $IgA_1$ and $IgA_2$ as well as innate factors including lactoferrin, lysozyme and lactoperoxidase [83]. Lung fluid total levels of IgG were higher in HIV

infected adults compared to controls and levels of pneumococcal capsule-specific IgG were not significantly different [80,84,85]. Capsule-specific IgG levels were highest in a group of HIV infected patients with recent invasive pneumococcal disease, suggesting that despite polyclonal IgG responses in HIV infected adults, appropriate responses to infection were also present [80]. Measurements of innate factors (lactoferrin, lysozyme, SPLI) were not different in BAL from HIV infected adults with or without recent pneumococcal disease compared to healthy adult control subjects (authors observations -manuscript submitted).

Phagocytic defects have been demonstrated in monocyte-derived macrophages and alveolar macrophages from HIV infected patients using fungi [86] and *Pneumocystis carinii* [87]. Complement-mediated and Fc-receptor mediated reticulo-endothelial clearance of radiolabelled erthyrocytes was noted to be reduced in HIV infected adults [88,89]. No defect in alveolar macrophage ingestion of opsonised *S.pneumoniae* type 1, coagulase-negative staphylococci or *Staphylococcus aureus* has been shown [90,91]. There are therefore, data to support both humoral and cellular immune defects in HIV-infected adults as well as evidence to suggest that important differences with functional consequences may exist between the humoral defense in pulmonary and circulating compartments.

### *Genetic Factors*

Alaskan natives [15], other native American peoples [92] and black Americans [93] are groups of people shown in epidemiological studies to have an excess of pneumococcal disease. The underlying host factors are beginning to be elucidated and include factors related to opsonisation including FcR polymorphism [94,95], CRP gene polymorphism [96] and MBL polymorphism [97]. Many more genetic factors are expected to emerge.

## PAST AND CURRENT PNEUMOCOCCAL VACCINES

Prevention of pneumococcal disease by vaccination has been a developing technique for the past 90 years [98]. Vaccines used to date have all depended on generating antibodies against capsular polysaccharide by systemic vaccination with polysaccharide of multiple serotypes. The 23-valent polysaccharide vaccine has been licensed since the early 1980's following studies undertaken in South African goldminers living in overcrowded conditions [99]. Subsequent randomised controlled trials conducted in other high risk groups have failed to reproduce the protective effect of polysaccharide vaccination in miners [74,100-104]. Retrospective analyses, however, have suggested that the polysaccharide vaccine provides some protection against invasive pneumococcal disease [93,105-109]. The capsular polysaccharide vaccine has not been shown to prevent pneumonia in adults or have any effect in children.

The development of protein-conjugate pneumococcal polysaccharide vaccines was driven by the need to protect children against pneumococcal disease. In the developed world this has included the desire to prevent mucosal pneumococcal infections in particular otitis media, as well as the more serious syndromes of pneumonia, bacteraemia and meningitis. Six studies using these vaccines have now been published, including one from the developing world, as shown in Table **1** [110-115]. To summarise these studies,

**Table 1. Prospective Randomised Clinical Trials to Date of Protein Conjugate Pneumococcal Polysaccharide Vaccine in Children. Figures Underlined Indicate Significance at or Greater than the 5% Level**

| | | | | | | Efficacy % | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Reference** | **Year** | **Sample Size** | **Duration Months** | **Site** | **Vaccine type** | **IPD[1]** | **Pnm[2]** | **OM[3]** | **Comments** |
| Black (110) | 00 | 37,868 | 43 | US | 7-CRM[4] | <u>94</u> | | <u>7</u> | |
| Eskola (111) | 01 | 1,662 | 17 | Finland | 7-CRM | - | - | 6 | 34% reduction in culture confirmed pneumococcal OM |
| Kilpi (112) | 00 | 1,666 | 18 | Finland | 7-OMPC[5] | - | - | -1 | 25% reduction in pneumococcal OM |
| O'Brien (113) | 03 | 8,292 | 37 | US | 7-CRM | <u>83</u> | - | - | |
| Veenhoven (114) | 03 | 383 | 18 | Netherlands | 7-CRM +23-PPV | - | - | - 25% | Combination vaccination as secondary prophylaxis in recurrent OM sufferers |
| Klugman (115) | 02 | 39,879 | 24 | SA | 9-CRM | <u>83</u> | <u>22</u> | - | 53% reduction in vaccine serotype IPD in sub-group of HIV-infected children |

[1] Invasive pneumococcal disease of vaccine serotype
[2] All cause pneumonia
[3] Acute otitis media
[4] Mutant diphtheria toxoid - CRM197 used as the protein carrier
[5] Meningococcal outer membrane protein complex used as the carrier

conjugate vaccines induce good serotype-specific immunity against invasive syndromes but more modest protection against mucosal infection. Unlike the older polysaccharide vaccine the conjugate vaccines do produce a mucosal response to systemic vaccination [116] and this is believed to underpin their ability to prevent mucosal disease.

Although the protein–polysaccharide conjugate vaccines represent a great leap forward in terms of protecting children problems with these vaccines remain.

- Current vaccines have limited serotype coverage and it is unclear what the maximum number of serotypes that can be incorporated into these vaccines will be. There are 90 serotypes of pneumococcus with varying distributions around the world. The currently available 7-valent conjugate vaccine is effective against 80-90% of disease in Europe and the USA but may only cover 25% of serotypes causing disease in southern Africa [117].
- Conjugate vaccines induce changes in the carried serotypes of pneumococci. This may result in a change over time of pneumococci causing mucosal and invasive syndromes necessitating a change in vaccine serotype formulation. Evidence exists for this effect in otitis media [111] but not yet for invasive infection.
- They are expensive. This is not simply a reflection on drug companies recouping drug development costs, but the vaccines are technically demanding to produce and will continue to have significant manufacturing costs.
- Vaccine-serotype specific efficacy against mucosal infections appears lower than against invasive infections. Mucosal infections contribute a much larger burden of disease than the invasive syndromes and their remains a need to reduce morbidity and the disability they create.
- There is no evidence to suggest these vaccines will be any more effective than the older polysaccharide vaccine in adult populations.

Continued development of pneumococcal vaccines remains essential. The evaluation of new vaccine candidates is on-going – the pneumococcal peptides pneumolysin and pneumococcal surface adhesin A discussed below are perhaps the most well-developed of these. However, in addition to the antigens used, the route by which we vaccinate needs investigation. It is tempting to believe that the most appropriate protective immune response against the pneumococcus, a respiratory pathogen, will be derived from vaccination of the respiratory mucosa [118]. Evidence from animal studies provides some support for this and whether these findings can be translated into humans now needs to be evaluated. This paper reviews current knowledge about anti-pneumococcal immunity and puts forward the case for investigating respiratory mucosal vaccination.

## HOST DEFENSE AGAINST PNEUMOCOCCAL DISEASE

Since optimal vaccination should augment natural defense against pneumococcal disease, a review of the pathogenesis of pneumococcal disease is relevant to this discussion, along with a discussion of the mechanisms of antibody response to pneumococcal components.

## PATHOGENESIS OF PNEUMOCOCCAL DISEASE

### Colonisation, Inhalation and Epithelial Binding

Asymptomatic nasopharyngeal carriage is established by binding between nasal epithelium and surface components of the pneumococcus [32,119]. Pneumococci of a given genotype exist in different phenotypes (termed transparent and opaque phases due to colony morphology on clear agar) dependent on conditions of oxygen tension [120]. The predominant pneumococcal phenotype in the nose is the transparent phase, which exhibits less polysaccharide capsule and more prominent surface proteins than the capsule-rich opaque phase. In the transparent phase, pneumococci exhibit increased binding to epithelium, increased colonisation in animal models and reduced resistance to host defence, particularly phagocytosis [121]. Nasopharyngeal colonisation typically ends after several months and this is accompanied by the detection of capsule specific mucosal and circulating antibody [122,123]. Adults experience less nasopharyngeal colonisation than children and are colonised by pneumococci with a smaller range of capsular types, likely due to the development of persistent capsule-specific mucosal antibody [124,125].

Inhaled pneumococci present a threat to the host as the lower respiratory epithelium lacks the barrier defenses of the upper airway [126]. Specifically, pneumococcal cell wall moieties have been shown to bind to the platelet-activating factor (PAF) receptor on alveolar epithelium [127]. Bound pneumococci are then internalised by epithelial cells, transported in a phase-dependent manner to the basal surface of the cell and extruded thus achieving epithelial penetration [128]. Mucosal disease provokes a predominantly local response, but invasive disease causes systemic inflammatory responses and an antibody response critically dependent on splenic function.

### Pulmonary Immune Responses to Pneumococci

Pulmonary immunity can be divided in to innate and acquired responses. Innate immunity is a system of genetically inherited defence mechanisms based on pathogen pattern recognition. Innate immunity serves both as a first line pathogen recognition and early response system and also as the means by which the acquired response is correctly focussed. The acquired immune system is found only in vertebrates and involves the response of B and T lymphocytes to produce an antigen-specific, amplified and more effective inflammatory response.

#### *Pulmonary Innate Immunity*

Pulmonary innate immune defenses interact with inhaled pneumococci in several ways. First, patrolling surface phagocytes, predominantly alveolar macrophages [129,130], ingest small numbers of inhaled bacteria and particles using a variety of surface receptors including scavenger receptors, mannose receptor, immunoglobulin and complement receptors. Ingestion of small numbers of particles or bacteria does not result in an inflammatory response due to the immunoregulatory properties of alveolar macrophages [131]. Second, pneumococci are recognised by several receptors including toll-like receptor 2 (TLR2) [132], which recognises Gram positive bacterial motifs such as

lipoteichoic acid, TLR9 which recognises non-methylated bacterial CpG DNA and the PAF receptor mentioned above. Toll-like receptors initiate $NF_{K}B$ dependent gene transcription by several mechanisms and this results in increased cytokine expression, increased bone-marrow release and increased migration of phagocytes, particularly neutrophils. *S.pneumoniae* has been shown to induce chemokine production by respiratory epithelium [133] and to induce early neutrophil migration from the marginated pulmonary intravascular pool to the alveolar space by a CD18 independent mechanism [134]. Resident alveolar effectors of innate immunity (likely alveolar macrophages) therefore, have a **critical period** in which to remove inhaled pneumococci before inflammatory signals are produced to recruit neutrophils. Alterations in pulmonary capillary neutrophil deformability have been shown to occur in less than 60 minutes after bacterial challenge. Recent work has demonstrated that the classical pathway is the dominant complement pathway required for innate immunity to *S.pneumoniae* in mice [54]. Complement activation is likely opsonic and pro-inflammatory in the lung. Inflammation resulting in pneumonia is initially compartmentalised within the affected lung as demonstrated by measurements of pulmonary and circulating cytokine levels [135].

### *Pulmonary Acquired Immunity*

In the lung, the acquired response has 3 distinct phases – afferent antigen processing and transport to regional lymph nodes [136], presentation to naïve lymphocytes and then efferent migration of activated T lymphocytes and mature B cells to the inflamed area of lung [137]. Pulmonary antigen presenting cells consist of dendritic cells [138] and B lymphocytes [139] as alveolar macrophages have weak antigen presenting properties [140,141]. Pulmonary acquired immune responses result in the presence of antigen-specific immunoglobulin in the lung lining fluid and consequent increased effectiveness of professional phagocytes such as alveolar macrophages and neutrophils [142]. In healthy adults, pneumococcal polysaccharide specific immunoglobulin has been shown to be present in bronchoalveolar lavage fluid (BAL) with increased levels detectable in patients with a recent history of pneumococcal disease [80].

## Pneumococcal Virulence Factors Cause Accelerated Inflammation

After pneumococcal infection is established on the mucosal surface, bacterial virulence factors (eg pneumolysin, neuraminidase) contribute to the rapid spread of bacteria in to the circulation [22,143]. Pneumococci invading the blood are predominantly opaque in phenotype as this phase are most resistant to phagocytosis [120]. The inflammatory process is rapidly accelerated by the fact that non-surface antigens of pneumococcus, including cell wall components and cytoplasmic antigens, cause rapid activation of complement and a pro-inflammatory response. This has been demonstrated in models of pneumococcal meningitis [144] and pneumonia using lysed bacterial products and is relevant to vaccine design since any cytoplasmic product is likely to induce overwhelming inflammation, even in the absence of infection [126].

The reticulo-endothelial system and in particular liver and spleen macrophages, has been demonstrated as being critical in opsonic removal of pneumococci and pneumococcal debris from the circulation [145]. Thus, defence against pneumococcaemia is critically dependent on immunoglobulin, complement and a functioning spleen. Asplenic patients are susceptible to overwhelming pneumococcal infection [56].

Meningitis and other tissue invasive pneumococcal infections occur when bacteraemia is unchecked for sufficiently long that endothelial layers can be crossed. In the case of the meninges, transparent phase pneumococci are able to bind endothelial receptors and are then transported to the CSF in a phase and receptor dependent manner [146].

## PULMONARY ANTIGEN PROCESSING AND ANTI-BODY PRODUCTION

Pneumococci present protein, polysaccharide and glycoprotein conjugates to the bronchial and alveolar surfaces. Protein or glycoprotein antigens are processed in a different manner to pure polysaccharides and each will therefore be described separately.

## Protein Antigen Processing

Alveolar macrophages comprise the majority of cells obtained at bronchoalveolar lavage (>90%) but these cells are relatively ineffective at antigen presentation [141,147] and are better considered as immunoregulatory cells of the alveolar surface [140,148]. Nevertheless, alveolar macrophages have been shown to ingest pneumococci *in vitro* [142] and antigen internalised by alveolar macrophages can be transferred to dendritic cells for antigen presentation [149,150]. Dendritic cells are considered to be the predominant antigen presenting cells in the lung [137]. These cells are a population of lung macrophages that are found predominantly in the submucosa [138,151]. They have many spindle-shaped processes, some of which impinge on the airspaces of the lung. Dendritic cells constantly sample the alveolar milieu by endocytosis of small particles, pinocytosis of soluble antigen and phagocytosis of particles greater than 1µm in diameter [151]. Dendritic cells in the lung parenchyma exhibit an immature phenotype which is ineffective at antigen presentation to lymphocytes [140], but on challenge with protein antigen and a critical innate immune stimulus ("danger signal"), immature dendritic cells migrate to the regional lymph node [136,137,152]. This is achieved under the influence of a chemotactic gradient and increased expression of CCR7 [153-155]. During migration, expression of MHC class II molecules is increased and the dendritic cell production of cytokine increases [151]. The cytokines produced are critically dependent on the innate immune danger signal received and will in turn influence the TH1 or TH2 nature of the germinal centre response (proliferation of antigen specific T and B cells) obtained at the regional lymph node [137,156]. At the lymph node, lymphocyte responses to antigen presented by dendritic cells are regulated by 3 signals - antigen presentation [157], co-receptor signals of both pro- and anti-inflammatory nature [158] and cytokine signals [151,159,160]. The germinal centre will persist as long as antigen is presented and will

result in B-cells maturing to plasma cells for export, memory cells that find a niche in the node and B-cells with inadequate affinity maturation which undergo apoptosis. Affinity maturation of B cell responses within the germinal centre of the regional lymph node is dependent on antigen signal in the context of co-receptor and cytokine signals [161,162]. Mature effector cells produced as a result of antigen presented in the regional lymph node traffic back to the inflamed region by means of the blood using high expression of chemokine receptors and possibly activated complement to localise the inflamed area [163-165]. This response will include mature B-cell derived plasma cells and T cells of both CD4 and CD8 lineage including immunosuppressive regulatory T cells ($T_{REG}$) [166]. The effector response that emerges at the mucosal surface is the result of a critical balance of these effectors and the presence or absence of persistent antigenic stimuli [167-169].

Not all antigen presented to dendritic cells results in an inflammatory response- when these cells are presented with antigen alone, in the absence of a danger signal detected by the innate immune system, they do not mature but instead induce tolerance to the antigen by an IL-10 dependent mechanism [151]. In addition, B cells in the regional lymph node which recognise antigen in the presence of T cell activation produce an effective response but presentation of either antigen alone or T cell activation in the absence of antigen produce B cell apoptosis [170,171].

## Polysaccharide Antigen Processing

Polysaccharide antigens do not elicit a response in the immature immune system of young children. There is much current research investigating the mechanisms by which a mature immune system can respond to polysaccharide antigen, predominantly using injected models of antigen presentation.

### *Polysaccharide Antigen Presentation- Systemic, Pulmonary, or Both?*

One intriguing facet of pulmonary studies is that the lung is both a mucosal surface in terms of histology and the production of secretory IgA and a potential site of absorption of peptides and other small molecules. This has led to the substantial interest in the use of the lung as a site for delivery of peptide hormones, hence avoiding the first pass effect due to liver metabolism. In the context of this review, however, antigen presented to the lung must be considered as potentially absorbed and so the distinction between injected and inhaled presentation of antigen is most likely the distinction between systemic only versus mucosal and systemic presentation.

Using injected polysaccharide models, B cells are of critical importance as antigen presenting cells in responses to polysaccharide antigens because pure polysaccharide is not presented in the MHC groove but is directly bound to the B cell receptor [172]. Furthermore, polysaccharide antigens are defined as T-independent antigens (TI) because polysaccharide can elicit an effective humoral immune response from B cells when injected into the nude (athymic therefore, T-cell deficient) mouse [8]. TI antigens are further subdivided into types 1 and 2 on the basis of their immunogenicity in CBA/N mice (an X-linked immunodeficiency resulting in defects in B cell receptor signalling) [162,173]. Pneumococcal polysaccharide is defined on this basis as a TI-2 antigen (no response in CBA/N mice) and has been shown to induce an antibody response in isolated human B cells *in vitro* [174]. It is important to emphasize, however, that the TI-2 laboratory definition of a polysaccharide antigen does not exclude a role for T cells *in vivo*. Indeed, a regulatory effect of T-cells on TI-2 responses has been demonstrated [166,175,176] and a modulating effect of CD40 antibodies (providing a surrogate for T cell help) has shown increased protective antibody production in a mouse model [177]. The adult human capacity to respond to carbohydrate antigen with the production of specific $IgG_2$ is likely the result of a mature $T_H$ cell population able to facilitate the production of antibody by selected B-cells presenting appropriate antigen [178,179] or appropriate idiotype antibody [176].

### *Possible Pulmonary Polysaccharide Antigen Presentation*

Pulmonary responses to polysaccharide antigen have not been fully described. Immature dendritic cells likely take up polysaccharide antigens presenting to the lung by pinocytosis or endocytosis according to whether the polysaccharide is soluble or in particulate form. Alveolar macrophages ingest small numbers of intact bacteria and may transfer antigen. Whole pneumococci have been shown to elicit both protein and polysaccharide responses from dendritic cells [180]. DC migrate to the regional lymph node and present antigen to extra-follicular B cells as with protein antigen [152,180] but it is not clear if this is a fully effective mechanism in TI-2 immunity. Lymph node B cells are not capable of responding alone to polysaccharide *in vitro* but produce an appropriate response in the presence of dendritic cells of splenic origin [35,181]. The known critical role of the spleen in antibody responses is due to the high concentration of particular populations of B cells (CD21 expressing and IgM memory cells) and macrophages which are together capable of making a rapid, effective antibody response [182]. It is not known if this response can be ellicited in pulmonary lymph nodes. These are important subjects for further investigation.

### *B Cell Responses to Pure Polysaccharide Antigens- Spleen Alone or Lymph Nodes Too?*

B cell responses to polysaccharide are elicited from 3 types of B cells –marginal zone splenic B cells, B1 cells and extra-follicular B cells [37,173]. The most rapid response, also producing the largest amount of antibody, is derived from marginal zone (MZ) B cells in the spleen. These are partially activated B cells as shown by their large size, co-receptor expression and preformed granular immunoglobulin. MZ B cells produce a rapid, short-lived response that results in a peak of circulating plasmablasts 3-7 days after antigen stimulation. B1 cells are found in the peritoneum and pleura and produce low levels of immunoglobulin to a restricted number of fixed patterns in a manner similar to the collectins produced by the innate immune system [183]. Persistent polysaccharide antigen will, however, elicit a specific response in these cells. Finally and of most interest in considering pulmonary responses, extra-follicular B cells in the medullary region of lymph nodes are potentially capable of responding to

polysaccharide antigen in the context of dendritic cell support [35]. Additionally, small numbers of MZ type B cells may be found in regional lymph nodes. Polysaccharide antigens do not produce classical germinal centres and as a result there is no B cell memory. The persistence of response, however, is determined by the duration of persistence of the antigen and there is some evidence that particulate antigen persists longer in the follicular dendritic cells [172]. Plasma cells that migrate back to the lung surface are likely short-lived (less than 3 months) but may be capable of producing an increased response when presented with further antigen [184]. It is of clinical relevance to note that some post-splenectomy patients can mount an effective response to pneumococcal vaccines.

### *Protein –Polysaccharide Conjugates*

Protein polysaccharide conjugates do bind MHC molecules and elicit specific T cell responses [185] and this provides the basis for the successful protein conjugated polysaccharide vaccines against *Haemophilus influenzae* and 7 serotypes of pneumococci. Unfortunately, the conjugate vaccines retain some TI-2 properties in that they do not show evidence of a booster effect [186], or affinity maturation in adults [187]. There are no data on antibody production in response to pulmonary challenge with conjugate vaccine.

## THE CASE FOR AN INHALED VACCINE

### Immunological Advantages of Mucosal Vaccine

The primary advantage of a mucosal vaccine is that the antigen is presented in a manner sufficiently similar to the invading pathogen that, as demonstrated in animal studies, maximally effective responses can be locally produced in the appropriate compartment [188-192]. Studies of human subjects in military camps have shown that exposed but non-infected recruits in a pneumonia outbreak developed serotype-specific antibody [123] suggesting that mucosal exposure could elicit a protective response in humans. This type of antibody response has subsequently been confirmed in human nasal inoculation with whole pneumococci [193,194]. The differences in rodent and human nasal lymphatic drainage [152] and the local regulation of antibody production shown in animal studies [195], show that distal airway antigen presentation will be required to elicit pulmonary responses in humans [196]. Protein conjugate anti-pneumococcal vaccine induces a mucosal (salivary) response in children that is effective against otitis media and alters pneumococcal carriage. This response might be further optimised by inhaled vaccine delivery due to the compartmentalisation of pulmonary immunoglobulin responses reviewed above.

The second major advantage of a mucosal vaccine is suggested by evidence that the mucosal response is relatively preserved in the important patient groups at risk for pneumococcal disease – namely young children, the elderly [197] and patients with HIV disease [82]. This suggests that a mucosal vaccine might be more effective than systemic vaccination.

A final and important immunological case for inhaled vaccine is that multiple antigens can be delivered simultaneously without loss of immunogenic effect [198-200].

## ADVANTAGES OF NEEDLE FREE VACCINATION

There are advantages to needle free vaccination independent and in addition to the immunological advantages of mucosal vaccination [201]. Needle free mucosal vaccination strategies under current investigation are oral, nasal and inhaled vaccines but transcutaneous and percutaneous jet vaccination are also under development. The important advantages of needle free vaccination are different in resource-rich and resource- deprived regions of the world and in situations of acute disease outbreak.

In resource-rich regions, multiple vaccines are now available for measles, mumps, rubella, pertussis, polio, varicella, tetanus, diphtheria and so on. This plethora of vaccines has increased the number of injections to which parents are asked to expose their children and compliance has become a problem which has recently been compounded as public anxiety about the advisability of multivalent vaccination (MMR) has risen. Needle free vaccination may therefore, increase compliance as well as reducing occupational risks from needle stick injury in health workers. All forms of needle free vaccination may be helpful in this setting.

In resource-poor regions, the limited availability of funds and skilled staff makes widespread use of even a selected number of vaccines (expanded EPI programme) very difficult. There is an increasing disparity between the number of vaccines available in resource-rich regions and the number in use in resource-poor regions. In addition, the paucity of hygienic clinical supplies makes the risk of needle-stick transmitted infection a major worry in resource-poor regions. Needle free vaccination offers the chance of reduced costs in needles, syringes and the need for skilled staff, therefore, potentially allowing greater EPI effectiveness. In resource-poor regions, needle free vaccination must be developed in such a way that it is cheaper than existing methods and, therefore, there has been an emphasis on developing nasal, oral and simple inhaled vaccines. In addition, needle free vaccination could reduce the risk of transmission of HIV, hepatitis B and hepatitis C viruses, both among vaccine recipients and health workers.

In acute disease outbreaks, rapid delivery needle free percutaneous vaccination technology would be an advantage. As in all settings vaccination would be of greatest benefit if the vaccine was also temperature stable, delivered without the need for electricity and immunogenic in less than three doses [201].

## EXISTING NEEDLE FREE VACCINES

Oral polio vaccine is well known but there are other needle free vaccines already available or in the late stages of development.

Inhaled measles vaccine was studied in South America and Southern Africa and the data show inhaled measles vaccine to be immunogenic and effective. Appropriate nebuliser technology is now being developed to make measles vaccination by inhalation widely available to

**Table 2. Current Vaccines or Vaccine Candidate Antigens Suitable for Consideration as Inhaled Vaccines**

| Candidate | Comment | Animal studies on mucosal effect | Refs |
|---|---|---|---|
| *23-valent pneumococcal polysaccharide* | No memory or boosting but possible persisting response due to maintenance on dendritic cell surface possible. | No | |
| *Protein-polysaccharide conjugate* | Presented in MHC II dependent manner and response may be manipulated with adjuvant use | No | |
| *Pneumococcal peptides* | MHC II presentation, so can be used with adjuvants. Inducement of allergy/inflammation possible concern | | |
| *PspA (pneumococcal surface protein A)* | Phase 1 trials in humans well tolerated and produced passive protection in mice | Trials in mouse studies. No adverse pulmonary outcomes. Protection shown when used intra-nasally. | 23, 189-90, 214-28 |
| *PsaA (Pneumococal surface adhesin A)* | A metal binding lipoprotein (not an adhesin) | Systemic and nasal vaccination of mice protects against carriage | 194, 229-39 |
| *PdB (pneumolysin toxoid)* | Toxoid lacks pro-inflammatory characteristics of pneumolysin | Protects against lethal infection in mice given systemically. | 22, 188, 213, 222, 224, 235, 240-43 |

children in resource-poor regions. Influenza vaccine can be delivered by the nasal route but does not yet produce an increased mucosal response to that achieved by injection.

Mucosal vaccination for HIV has been an important research area and although a protective vaccine in humans has not yet been developed, important lessons have been learned and a degree of protection achieved in animal models [202]. In particular, it has been appreciated that mucosal immunity requires a coordinated response from innate and acquired immune effector cells. This involves appropriate antigen processing particularly by dendritic cells, followed by T-lymphocyte activation and accessory cell function and finally B cell migration resulting in local, specific and mucosal immunoglobulin. Needle free vaccination for hepatitis B virus has been studied using CpG DNA as an adjuvant [203], for *Mycobacterium tuberculosis* using sub-unit antigens presented to the gastic mucosa [204], for *Moraxella catarrhalis* using detoxified lipopolysaccharide presented to the nasal mucosa [205], for *Bordetella pertussis* using pulsed dendritic cells [206] and for various pneumococcal antigens reviewed below.

## MECHANISMS AND CANDIDATE ANTIGENS FOR INHALED ANTI-PNEUMOCOCCAL VACCINE

### Mechanisms for An Inhaled Vaccine

An inhaled vaccine must either contain pneumococci in the colonising but not invading state, or prevent colonisation altogether. Prevention of disease will involve boosted mucosal defense and a mechanism to prevent inhaled bacteria breaching the respiratory epithelium. This is most likely to be achieved by augmenting the existing respiratory defences reviewed above and in particular, by enhancing opsonin-enhanced phagocytosis of pneumococci by resident and transient phagocytes. The critical interval for pneumococcal disease is probably of the order of 4 hours since this is the time taken for transparent phase pneumococci to cross an epithelial barrier using a PAF receptor dependent mechanism [146].

## CANDIDATE ANTIGENS FOR AN INHALED VACCINE

Antigens that could potentially be useful in an inhaled vaccine against pneumococcal disease include all components of *Streptococcus pneumoniae* and the components of the inflammatory response that facilitate pneumococcal invasion [143]. The ideal mucosal vaccine would not impair barrier defence (mucous, ciliary function, epithelial integrity), but would be optimally taken up, processed and presented by antigen presenting cells (ideally dendritic cells) that migrate to a suitable location for interaction with naïve B and T cells (ideally the regional lymph node). Following optimal interaction between DC and lymphocytes, including cytokine production by accessory cells to ensure the correct TH1/TH2 balance in terms of response [157], maturing B cells must migrate to the appropriate mucosal compartment and produce effective antibody in the absence of a chronic inflammatory or allergic response. Finally, effective phagocytes must be able to act in the time-frame available between surface adhesion of the bacteria and uptake into intracellular or submucosal compartments. An additional factor to consider is the anti-inflammatory nature of the alveolar and airways cytokine and cellular milieu [207]. This regulated environment extends to the whole mucosal compartment and presents a particular problem as most antigens presented to the mucosal compartment fail to elicit an immunological response at all. Successful use of adjuvants in development of mucosal vaccines has been hampered by the excessive side effects of these products [208].

Candidate antigens can be selected from among pathogenicity factors of *S.pneumoniae* using observations of the immune response to these factors in recuperating patients

[209,210]. Surface moieties of pneumococcus are favoured as they are non-inflammatory [25,211]. Cell wall fragments and cytoplasmic products are pro-inflammatory [212] unless modified but one such product, a pneumolysin derivative (PdB), has been shown to be non-inflammatory and protective in an animal model [213]. Combinations of antigens may be more effective than single antigen vaccine [198-200].

The most promising inhaled vaccine candidates include **currently licensed vaccines** (polysaccharide and conjugate) and **novel peptide vaccines** that will elicit T-cell memory in the lung (Table **2**). Peptide vaccines include pneumococcal surface protein A (PspA), pneumococcal surface adhesin A (PsaA) and PdB. **PspA** is a virulence determinant [23,214] of all serotypes of pneumococci [215] and the protein has been described [216,217], cloned and produced for experimental work in recombinant systems [218,219]. PspA is immunogenic in humans [220] and protective against disease when injected or presented nasally or orally in animal models [221,222]. The immunogenicity of nasal presentation has been increased by co-presentation with IL-12 [189,223] or mutant cholera toxin [190]. Anti-PspA antibody responses in serum can be found in serum and saliva after IPD, otitis media or nasal carriage in humans [224-227]. Phase 1 trials of PspA as a candidate vaccine antigen have shown the test antigen to be immunogenic in humans and human antibody has protective efficacy against pneumococcal challenge in passive transfer experiments using mice [220,228]. Toxicity data remain to be evaluated. **PsaA** is a metal-binding lipoprotein shown to be a virulence determinant of all 90 serotypes of pneumococci [229,230]. The structure of this protein has been described [231] and the gene cloned allowing protein production by recombinant technology in sufficient quantities for experimental work [232]. PsaA antibodies are found in humans after recovery from pneumonia [233], otitis media [234] and invasive pneumococcal disease [224,226] and are induced by nasal carriage of pneumococci [194]. PsaA-specific B cells have been recovered from human adenoidal lymphoid tissue and further challenge of adenoidal cells *in vitro* produced increased responses suggestive of a booster effect [235]. Injected PsaA elicited a protective response against pneumococcal carriage [236] and invasive disease in mice [237] and a PsaA DNA vaccine is under development [238]. Oral immunisation using PsaA in microspheres produced protection in the respiratory tract in mouse models [239]. Phase 1 trials in humans have not yet been published. **PdB** is a non-toxic derivative [213] of pneumolysin [22,240]. Antibodies against pneumolysin are found in humans following pneumonia [241], otitis media [242] and invasive disease [224] and anti-pneumolysin antibodies were found in BAL following challenge with whole dead pneumococci in animal experiments [188]. Injected PdB was protective against disease in animal models [222,243] and PdB-specific cells were recovered from adenoidal tissue, albeit at lower levels than PsaA specific cells [235]. Phase 1 trials in humans have not yet been published.

### Adjuvants

Mucosal surfaces are poorly responsive to antigen [140,141] and this property protects the host from excessive inflammation in response to inhaled air or food. Mucosal vaccination as a strategy has been delayed by the hypo-responsiveness of the compartment in the absence of an adjuvant and by the toxicity of available adjuvants. Adjuvants to optimise mucosal vaccine immunogenicity have been developed in animal models [200,208] and several now have low toxicity sufficient to consider human use [191,244,245]. Cholera toxin (CT) and the heat labile enterotoxin (LT) of *E.coli* have been demonstrated to be effective adjuvants, particularly at very low doses, but cause extreme diarrhoea. More recently, the B subunit of CT (CT-B) [246], a non-toxic CT-A subunit [190] and modified LT variants (LTK63 and LTR72) [200] have been shown to retain effective adjuvant activity in the absence of side-effects. Importantly, the dose-response studies of these adjuvants indicate that they are effective at microgram doses and towards several antigens at once [200]. It has been proposed that the adjuvant activity is mediated by a CD80 and CD86 mechanism [190].

## POTENTIAL PITFALLS: DRUG DELIVERY, IMMUNOGENICITY, SAFETY AND EFFICACY

### Drug Delivery

It is critical in mucosal immunisation that the effector response is targeted to the intended mucosal surface. In animal studies, antigen challenge has demonstrated local immune responses localised to a single lobe of lung [247]. Inhaled vaccine candidate antigens must therefore, reach the catchment area of all significant local lymph nodes. In practice, this means that the delivered particles must be approximately 1μm in diameter and delivered at low speed by nebuliser or other breath-activated device to a subject using tidal breathing. For development purposes, nebulised antigen solutions will allow greatest control of total dose at high dilution but eventually a breath-activated dry powder device may be optimal in order to escape the need for a cold chain and electricity. Modern piezo-electric nebulisers offer portable, battery operated, low volume and reliable nebulised delivery of most solutions.

### Immunogenicity

Immunogenicity is critically altered by antigen dose, co-receptor expression and cytokine milieu. Antigen excess may result in anergy due to a lack of DC stimulation by innate immune signals. This is the principle behind desensitisation therapy in allergy and is a potential problem for effective vaccination. Immunogenicity will be maintained by correct antigen dose, co-receptors and cytokine responses.

Lack of critical co-receptors (or even antigen presenting cells) may explain the lack of response in patient groups at particular risk of pneumococcal disease. Lack of certain B cell subgroups in young infants may make these infants as unresponsive to pulmonary vaccination as to the currently available injected vaccines [37]. HIV infection may interfere with dendritic cell function by a DC-SIGN mediated down-regulation of antigen presentation and CD80 and CD86 co-receptor expression [66].

Cytokine balance is critical in determining the Th1/Th2 balance of the immune response to antigen [169]. More

recent understanding has extended this model to include Th0, Treg and Thpp pools of lymphocytes. It may be that HIV infected patients, for example, have an altered cytokine milieu that interferes with optimal antigen processing in the lung or presentation in the lymph node. Alternatively, the known pro-inflammatory effects of inhaled pollution [248-250] may interfere in vaccine efficacy, just as particulate exposure is noted to be associated with increased risk of respiratory infection [251,252].

### Safety

Immune regulation by vaccination has great potential benefits in life saved and morbidity avoided but necessarily involves a degree of risk. The pro-inflammatory risks in presenting antigen aimed at altering pulmonary immunity are acute lung injury, asthma, allergy, anaphylaxis and pulmonary fibrosis. There is also a small risk of inducing an excessive anti-inflammatory response leading to anergy to the immunised antigen.

Acute lung injury or nasal inflammation have not been observed in animal models or in human studies using surface components of pneumococci, live capsulate pneumococci or unencapsulated pneumococci. Inflammation has arisen in animal models when complement fixing antigens such as cytoplasmic products (eg pneumolysin), or cell wall products (eg lipoteichoic acid) were used.

Asthma has not been induced in inhalational studies to date but can be easily exacerbated in mild asthmatic or latent atopic individuals [253]. These asthmatic responses can be early or late and are familiar to respiratory physicians with training in occupational medicine and inhalational challenge as a diagnostic tool [254]. There is a theoretical risk that protein antigens presented with adjuvant could result in a Th2 response of sufficient strength that a secondary exposure would result in an allergic response. This is unlikely following a single exposure because the common asthma allergens (house dust mite, animal dander and occupational hazards) require prolonged exposure and are among the most ubiquitous allergens to which humans are exposed. In any antigen exposure, anaphylaxis is a theoretical risk that must be minimised by appropriately cautious experimental protocols and close monitoring in a medically safe environment.

Pulmonary fibrosis is often idiopathic but in cases where the cause is known (eg asbestosis, farmer's lung) the exposure has been incurred for many years. In addition, in cases where pulmonary fibrosis occurs as part of an inflammatory condition (eg sarcoidosis, ankylosing spondylitis), the inflammatory condition has also typically been present for many years. It therefore, seems unlikely that a small number of inhalational challenges with candidate vaccines will induce pulmonary fibrosis.

### Memory

Finally, an effective antibody response lasting only a matter of weeks would be of little clinical benefit. Polysaccharide antigens are known to produce a short-lived (3 months), oligoclonal response with no booster or memory effect. An effective vaccine must also induce a memory response both in B cells (plasma cells producing antibody at the mucosal surface for at least 5 years) and in the T cell population required for B cell support (ideally a life-long response but at least 10 years). The duration of effect of protein conjugate anti-pneumococcal vaccines is not yet known, but may be limited by changes in carried serotype epidemiology. Ideally, immunity should be life-long and this is possible with peptide inhaled vaccine candidates as natural colonisation or exposure should provide a booster effect.

## MODULATION OF THE PULMONARY IMMUNE RESPONSE TO OPTIMISE AN INHALED VACCINE

Some of the potential pitfalls in designing an inhaled vaccine have been detailed above. Recent advances in our understanding of pulmonary immunology and immunotherapy, however, may allow manipulation of the pulmonary response to counteract these problems. In particular, antigen size manipulation, co-receptor replacement and cytokine manipulation may critically alter the innate response to antigen and facilitate an optimal acquired response.

### Systemic Priming

Conjugate vaccines produce mucosal responses with measurable levels of IgA in saliva and changes in pneumococcal carriage. Using an inhaled vaccine to boost mucosal protection may be an effective method of maintaining good sytemic response whilst producing the maximal mucosal response, but using the same vaccine antigen and avoiding adjuvants.

### Antigen Presentation - Particulate Suspensions or Solutions?

Polysaccharide and protein-polysaccharide conjugate responses are short-lived perhaps due to failure of memory induction in the primary response. One form of B cell memory is antigen-dependent memory that can be induced by persistent antigen. There is some evidence that particulate polysaccharide antigen is retained on the surface of dendritic cells in lymph nodes [172]. Therefore, nebulised suspensions of polysaccharide or protein-polysaccharide conjugates may result in more prolonged immune responses than antigens presented in solution.

### Co-Receptor Manipulation

Presentation of pneumococcal antigen to mice in the presence of CD40L was effective in preventing B cell apoptosis in a mouse model [177]. In addition, HIV has been shown to downregulate CD80 and CD86 expression in dendritic cells. Perhaps that can be replaced. There is evidence that adjuvants work by increasing CD80/86 expression and so this may be effective in HIV.

### Cytokine Manipulation

Patient therapy with either cytokine (eg IL-2, IL-12, IFN- ) or blocking monoclonal antibodies (anti-TNF) is now established [255,256] and further experimental strategies involving IL-10 [257,258], IL-11 [259], IL-18 [260], gene therapy [261,262] and dendritic cells [263] are under development. It is therefore, possible that co-presentation of

antigen with an appropriate cytokine (eg IL-12 or IL-18) may optimise the T cell response and lean towards an effective anti-pneumococcal Th1 response. These manipulations are some way off, however, as basic descriptions of response to antigen alone have not been carried out in human volunteers.

## CONCLUSION

Substantial recent progress in our understanding of mucosal biology, B cell immunology and the pathogenesis of pneumococcal disease suggest that a mucosal vaccine may be the best approach for prophylaxis against pneumonia and invasive disease in adult risk groups. Progress in pulmonary pharmacology has made the development of a needle-free vaccine possible. Promising candidate antigens have been developed in animal studies and are now ready for human trials. This is an important new field of investigation that will have important implications both within and beyond the prevention of pneumococcal disease.

## REFERENCES


[1] Hausdorff, W. P.; Bryant, J.; Paradiso, P. R.; Siber, G. R. *Clin. Infect. Dis.* **2000,** *30*(1), 100-121.

[2] Gray, B. M.; Converse, G. M.; Dillon-HC, J. *J. Infect. Dis.* **1980,** *142*(6), 923-933.

[3] Marrie, T. J. *Clin. Infect. Dis.* **2000,** *31*(4), 1066-1078.

[4] Nuorti, J. P.; Butler, J. C.; Gelling, L.; Kool, J. L.; Reingold, A. L.; Vugia, D. J. *Ann. Inter. Med.* **2000,** *132*(3), 182-190.

[5] Gordon, S. B.; Chaponda, M.; Walsh, A. L.; Whitty, C. J.; Gordon, M. A.; Machili, C. E.; Gilks, C. F.; Boeree, M. J.; Kampondeni, S.; Read, R. C.; Molyneux, M. E. *AIDS* **2002,** *16*(10), 1409-1417.

[6] Gilks, C. F.; Ojoo, S. A.; Ojoo, J. C.; Brindle, R. J.; Paul, J.; Batchelor, B. I.; Kimari, J. N.; Newnham, R.; Bwayo, J.; Plummer, F. A.; Warrell, D. A. *Lancet* **1996,** *347* 718-723.

[7] Givon-Lavi, N.; Fraser, D.; Porat, N.; Dagan, R. *J. Infect. Dis.* **2002,** *186*(11), 1608-1614.

[8] AlonsoDeVelasco, E.; Verheul, A. F.; Verhoef, J.; Snippe, H. *Microbiol. Rev.* **1995,** *59*(4), 591-603.

[9] Kalima, P.; Emmanuel, F. X.; Riordan, T. *Epidemiol. Infect.* **1999,** *122*(2), 251-257.

[10] Kim, P. E.; Musher, D. M.; Glezen, W. P.; Rodriguez, B. M.; Nahm, W. K.; Wright, C. E. *Clin. Infect. Dis.* **1996,** *22*(1), 100-106.

[11] Nuorti, J. P.; Butler, J. C.; Farley, M. M.; Harrison, L. H.; McGeer, A.; Kolczak, M. S.; Breiman, R. F. *N. Engl. J. Med.* **2000,** *342*(10), 681-689.

[12] Vold, P. P.; Owens, D. K. *Clin. Infect. Dis.* **2000,** *30*(1), 157-164.

[13] Smit, P.; Oberholzer, D.; Hayden Smith, S.; Koornhof, H. J.; Hilleman, M. R. *JAMA* **1977,** *238*(24), 2613-2616.

[14] Grobbelaar, J. P.; Bateman, E. D. *Thorax* **1991,** *46*(5), 334-340.

[15] Davidson, M.; Parkinson, A. J.; Bulkow, L. R.; Fitzgerald, M. A.; Peters, H. V.; Parks, D. J. *J. Infect. Dis.* **1994,** *170*(2), 368-376.

[16] Riley, I. D.; Tarr, P. I.; Andrews, M.; Pfeiffer, M.; Howard, R.; Challands, P.; Jennison, G. *Lancet* **1977,** *1*(8026), 1338-1341.

[17] Riley, I. D.; Lehmann, D.; Alpers, M. P. *Rev. Infect. Dis.* **1991,** *13* (Suppl. 6), S535-S541.

[18] Griffith, F. *J. Hyg.* **1928,** *27* 113-159.

[19] Scott, J. A.; Hall, A. J.; Dagan, R.; Dixon, J. M.; Eykyn, S. J.; Fenoll, A.; Hortal, M.; Jette, L. P.; Jorgensen, J. H.; Lamothe, F.; Latorre, C.; Macfarlane, J. T.; Shlaes, D. M.; Smart, L. E.; Taunay, A. *Clin. Infect. Dis.* **1996,** *22*(6), 973-981.

[20] Alonso-de-Velasco, E.; Dekker, B. A.; Verheul, A. F.; Feldman, R. G.; Verhoef, J.; Snippe, H. *J. Infect. Dis.* **1995,** *172* 562-565.

[21] Kengatharan, K. M.; De Kimpe, S.; Robson, C.; Foster, S. J.; Thiemermann, C. *J. Exp. Med.* **1998,** *188*(2), 305-315.

[22] Cockeran, R.; Anderson, R.; Feldman, C. *Curr. Opin. Infect. Dis.* **2002,** *15*(3), 235-239.

[23] Ren, B.; Szalai, A. J.; Thomas, O.; Hollingshead, S. K.; Briles, D. E. *Infect. Immun.* **2003,** *71*(1), 75-85.

[24] Yu, J.; Briles, D. E.; Englund, J. A.; Hollingshead, S. K.; Glezen, W. P.; Nahm, M. H. *J. Infect. Dis.* **2003,** *187*(6), 1019-1023.

[25] Briles, D. E.; Hollingshead, S.; Brooks-Walter, A.; Nabors, G. S.; Ferguson, L.; Schilling, M.; Gravenstein, S.; Braun, P.; King, J.; Swift, A. *Vaccine* **2000,** *18*(16), 1707-1711.

[26] Tuomanen, E.; Pollack, H.; Parkinson, A.; Davidson, M.; Facklam, R.; Rich, R.; Zak, O. *J. Infect. Dis.* **1988,** *158*(1), 36-43.

[27] Berry, A. M.; Lock, R. A.; Paton, J. C. *J. Bacteriol.* **1996,** *178*(16), 4854-4860.

[28] Duane, P. G.; Rubins, J. B.; Weisel, H. R.; Janoff, E. N. *Infect. Immun.* **1993,** *61*(10), 4392-4397.

[29] Wani, J. H.; Gilbert, J. V.; Plaut, A. G.; Weiser, J. N. *Infect. Immun.* **1996,** *64*(10), 3967-3974.

[30] Ricciardi-Castagnoli, P.; Granucci, F. *Nat. Rev. Immunol.* **2002,** *2*(11), 881-889.

[31] Stears, R. L.; Martinsky, T.; Schena, M. *Nat. Med.* **2003,** *9*(1), 140-145.

[32] Tuomanen, E. I.; Masure, H. R. *Microb. Drug Resist.* **1997,** *3*(4), 297-308.

[33] Meli, D. N.; Christen, S.; Leib, S. L.; Tauber, M. G. *Curr. Opin. Infect. Dis.* **2002,** *15*(3), 253-257.

[34] Koedel, U.; Scheld, W. M.; Pfister, H. W. *Lancet Infect. Dis.* **2002,** *2*(12), 721-736.

[35] Peset Llopis, M. J.; Harms, G.; Hardonk, M. J.; Timens, W. *J. Allergy Clin. Immunol.* **1996,** *97*(4), 1015-1024.

[36] Griffioen, A. W.; Toebes, E. A.; Zegers, B. J.; Rijkers, G. T. *Cell Immunol.* **1992,** *143*(1), 11-22.

[37] Kruetzmann, S.; Rosado, M. M.; Weber, H.; Germing, U.; Tournilhac, O.; Peter, H. H.; Berner, R.; Peters, A.; Boehm, T.; Plebani, A.; Quinti, I.; Carsetti, R. *J. Exp. Med.* **2003,** *197*(7), 939-945.

[38] Oldfield, S.; Jenkins, S.; Yeoman, H.; Gray, D.; MacLennan, I. C. *Clin. Exp. Immunol.* **1985,** *61*(3), 664-673.

[39] Garg, M.; Kaplan, A. M.; Bondada, S. *J. Immunol.* **1994,** *152*(4), 1589-1596.

[40] Carson, P. J.; Nichol, K. L.; O'Brien, J.; Hilo, P.; Janoff, E. N. *Arch. Inter. Med.* **2000,** *160*(13), 2017-2024.

[41] Feikin, D. R.; Schuchat, A.; Kolczak, M.; Barrett, N. L.; Harrison, L. H.; Lefkowitz, L.; McGeer, A.; Farley, M. M.; Vugia, D. J.; Lexau, C.; Stefonek, K. R.; Patterson, J. E.; Jorgensen, J. H. *Am. J. Pub. Health* **2000,** *90*(2), 223-229.

[42] Afessa, B.; Greaves, W. L.; Frederick, W. R. *Clin. Infect. Dis.* **1995,** *21*(2), 345-351.

[43] Garau, J.; Aguilar, L.; Rodriguez-Creixems, M.; Dal re, R.; Perez-Trallero, E.; Rodriguez, M.; Bouza, E. *J. Chemother.* **1999,** *11*(4), 266-272.

[44] Busse, W. W. *Rev. Infect. Dis.* **1991,** *13*(Supp. 6), S477-485.

[45] Janoff, E. N.; Rubins, J. B. Invasive pneumococcal disease in the immunocompromised host, in *Streptococcus pneumoniae: Molecular Biology and Mechanisms of Disease*, 1 ed.; Tomasz, A., editor; Mary Ann Liebert: New York, **2000**; pp. 321-341.

[46] Jones, N.; Huebner, R.; Khoosal, M.; Crewe-Brown, H.; Klugman, K. *AIDS* **1998,** *12*(16), 2177-2184.

[47] Usinger, W. R.; Lucas, A. H. *Infect. Immun.* **1999,** *67*(5), 2366-2370.

[48] Van der Hilst, J. C.; Smits, B. W.; van der Meer, J. W. *Neth. J. Med.* **2002,** *60*(3), 140-147.

[49] Amber, I. J.; Gilbert, E. M.; Schiffman, G.; Jacobson, J. A. *Transplant.* **1990,** *49*(1), 122-125.

[50] Goldfarb, N. S.; Avery, R. K.; Goormastic, M.; Mehta, A. C.; Schilz, R.; Smedira, N.; Pien, L.; Haug, M. T.; Gordon, S. M.; Hague, L. K.; Dresing, J. M.; Evans-Walker, T.; Maurer, J. R. *Transplantation* **2001,** *71*(2), 242-246.

[51] Hammarstrom, V.; Pauksen, K.; Svensson, H.; Lonnqvist, B.; Simonsson, B.; Ringden, O.; Ljungman, P. *Transplantation* **2000,** *69*(8), 1582-1586.

[52] Kazancioglu, R.; Sever, M. S.; Yuksel-Onel, D.; Eraksoy, H.; Yildiz, A.; Celik, A. V.; Kayacan, S. M.; Badur, S. *Clin. Transplant.* **2000,** *14*(1), 61-65.

[53] Alper, C. A.; Colten, H. R.; Rosen, F. S.; Rabson, A. R.; Macnab, G. M.; Gear, J. S. *Lancet* **1972,** *2*(7788), 1179-1181.

[54] Brown, J. S.; Hussell, T.; Gilliland, S. M.; Holden, D. W.; Paton, J. C.; Ehrenstein, M. R.; Walport, M. J.; Botto, M. *Proc. Natl. Acad. Sci. U.S.A.* **2002,** *99*(26), 169-174.

[55] Primack, W. A.; Rosel, M.; Thirumoorthi, M. C.; Fleischmann, L. E.; Schiffman, G. *Lancet* **1979,** *2*(8153), 1192.

[56] Winkelstein, J. A.; Drachman, R. H. *N. Engl. J. Med.* **1968,** *279*(9), 459-466.

[57] Onwubalili, J. K. *J. Infect.* **1983,** *7*(1), 2-20.
[58] Janoff, E. N.; Rubins, J. B. *Microb. Drug Resist.* **1997,** *3*(3), 215-232.
[59] Rowland-Jones, S. L. *Nat. Rev. Immunol.* **2003,** *3*(4), 343-348.
[60] Zamarchi, R.; Barelli, A.; Borri, A.; Petralia, G.; Ometto, L.; Masiero, S.; Chieco-Bianchi, L.; Amadori, A. *AIDS* **2002,** *16*(9), 1217-1226.
[61] Bergamini, A.; Bolacchi, F.; Bongiovanni, B.; Colizzi, V.; Cappelli, G.; Uccella, I.; Cepparulo, M.; Capozzi, M.; Mancino, G.; Rocchi, G. *J. Infect. Dis.* **2000,** *182*(3), 776-784.
[62] Olivetta, E.; Percario, Z.; Fiorucci, G.; Mattia, G.; Schiavoni, I.; Dennis, C.; Jager, J.; Harris, M.; Romeo, G.; Affabris, E.; Federico, M. *J. Immunol.* **2003,** *170*(4), 1716-1727.
[63] Twigg, H. L. 3.; Iwamoto, G. K.; Soliman, D. M. *J. Immunol.* **1992,** *149* 1462-1469.
[64] Mirani, M.; Elenkov, I.; Volpi, S.; Hiroi, N.; Chrousos, G. P.; Kino, T. *J. Immunol.* **2002,** *169*(11), 6361-6368.
[65] Mathys, J. M.; Melanson, S. M.; Schiffer-Alberts, D. J.; Ioannidis, J. P.; Koziel, H.; Skolnik, P. R. *J. Immunol.* **2000,** *164*(3), 1588-1594.
[66] Lore, K.; Sonnerborg, A.; Brostrom, C.; Goh, L. E.; Perrin, L.; McDade, H.; Stellbrink, H. J.; Gazzard, B.; Weber, R.; Napolitano, L. A.; van Kooyk, Y.; Andersson, J. *AIDS* **2002,** *16*(5), 683-692.
[67] Izmailova, E.; Bertley, F. M.; Huang, Q.; Makori, N.; Miller, C. J.; Young, R. A.; Aldovini, A. *Nat. Med.* **2003,** *9*(2), 191-197.
[68] Mascart, L. F.; Gerard, M.; Libin, M.; Crusiaux, A.; Franchioly, P.; Lambrechts, A.; Goldman, M.; Clumeck, N. *J. Infect. Dis.* **1995,** *172*(5), 1253-1260.
[69] Amdahl, B. M.; Rubins, J. B.; Daley, C. L.; Gilks, C. F.; Hopewell, P. C.; Janoff, E. N. *Am. J. Respir. Crit. Care Med.* **1995,** *152*(6 Pt 1), 2000-2004.
[70] Chang, Q.; Abadi, J.; Alpert, P.; Pirofski, L. *J. Infect. Dis.* **2000,** *181*(4), 1313-1321.
[71] Subramaniam, K. S.; Segal, R.; Lyles, R. H.; Rodriguez-Barradas, M. C.; Pirofski, L. A. *J. Infect. Dis.* **2003,** *187*(5), 758-768.
[72] King, J. C. J.; Borkowsky, W.; Mahidhara, N.; Madore, D.; Shapiro, E. D.; Rutstein, R. M.; Tan, T. Q.; Farley, J. J.; Dankner, W. M.; Nachman, S.; Simoes, E.; Flynn, P. M.; Clemens, J.; Hamilton, R. G. *J. Infect. Dis.* **2000,** *181*(5), 1817-1821.
[73] Janoff, E. N.; Douglas-JM, J.; Gabriel, M.; Blaser, M. J.; Davidson, A. J.; Cohn, D. L.; Judson, F. N. *J. Infect. Dis.* **1988,** *158*(5), 983-990.
[74] French, N.; Nakiyingi, J.; Carpenter, L. M.; Lugada, E.; Watera, C.; Moi, K.; Moore, M.; Antvelink, D.; Mulder, D.; Janoff, E. N.; Whitworth, J.; Gilks, C. F. *Lancet* **2000,** *355*(9221), 2106-2111.
[75] French, N.; Gilks, C. F.; Mujugira, A.; Fasching, C.; O'Brien, J.; Janoff, E. N. *AIDS* **1998,** *12*(13), 1683-1689.
[76] Musher, D. M.; Phan, H. M.; Watson, D. A.; Baughn, R. E. *J. Infect. Dis.* **2000,** *182*(1), 158-167.
[77] Janoff, E. N.; O'Brien, J.; Thompson, P.; Ehret, J.; Meiklejohn, G.; Duvall, G.; Douglas-JM, J. *J. Infect. Dis.* **1993,** *167*(1), 49-56.
[78] Janoff, E. N.; Fasching, C.; Ojoo, J. C.; O'Brien, J.; Gilks, C. F. *J. Infect. Dis.* **1997,** *175*(4), 975-978.
[79] Janoff, E. N.; Hardy, W. D.; Smith, P. D.; Wahl, S. M. *J. Immunol.* **1991,** *147*(7), 2130-2135.
[80] Gordon, S. B.; Miller, D. E.; Day, R. B.; Ferry, T.; Wilkes, D. S.; Schnizlein-Bick, C. T.; Zijlstra, E. E.; Read, R. C.; Molyneux, M. E.; Twigg, H. L. III. *J. Infect. Dis.* **2003,** *188*(5), 666-670.
[81] Moja, P.; Jalil, A.; Quesnel, A.; Perol, M.; Cotte, L.; Livrozet, J. M.; Boibieux, A.; Chamson, A.; Vergnon, J. M.; Lucht, F.; Tran, R.; Pozzetto, B.; Genin, C. *Clin. Exp. Immunol.* **1997,** *110*(3), 341-348.
[82] Scamurra, R. W.; Nelson, D. B.; Lin, X. M.; Miller, D. J.; Silverman, G. J.; Kappel, T.; Thurn, J. R.; Lorenz, E.; Kulkarni-Narla, A.; Janoff, E. N. *J. Immunol.* **2002,** *169*(7), 4008-4016.
[83] Opstad, N. L.; Daley, C. L.; Thurn, J. R.; Rubins, J. B.; Merrifield, C.; Hopewell, P. C.; Janoff, E. N. *J. Infect. Dis.* **1995,** *172*(2), 566-570.
[84] Fahy, R. J.; Hart, J.; Bees, T.; Diaz, P.; Wewers, M.D. *Am. J. Respir. Crit. Care Med.* **1999**, *159*(3), A753.
[85] Fahy, R. J.; Diaz, P. T.; Hart, J.; Wewers, M. D. *Chest* **2001,** *119*(1), 196-203.
[86] Ieong, M. H.; Reardon, C. C.; Levitz, S. M.; Kornfeld, H. *Am. J. Respir. Crit. Care Med.* **2000,** *162*(3 Pt 1), 966-970.
[87] Koziel, H.; Li, X.; Armstrong, M. Y.; Richards, F. F.; Rose, R. M. *Am. J. Respir. Cell Mol. Biol.* **2000,** *23*(4), 452-459.
[88] Bender, B. S.; Bohnsack, J. F.; Sourlis, S. H.; Frank, M. M.; Quinn, T. C. *J. Clin. Invest.* **1987,** *79*(3), 715-720.
[89] Bender, B. S.; Frank, M. M.; Lawley, T. J.; Smith, W. J.; Brickman, C. M.; Quinn, T. C. *J. Infect. Dis.* **1985,** *152*(2), 409-412.
[90] Musher, D. M.; Watson, D. A.; Nickeson, D.; Gyorkey, F.; Lahart, C.; Rossen, R. D. *Am. J. Med. Sci.* **1990,** *299*(3), 158-163.
[91] Gordon, S. B.; Molyneux, M. E.; Boeree, M. J.; Kanyanda, S.; Chaponda, M.; Squire, S. B.; Read, R. C. *J. Infect. Dis.* **2001,** *184*(10), 1345-1349.
[92] Benin, A. L.; O'Brien, K. L.; Watt, J. P.; Reid, R.; Zell, E. R.; Katz, S.; Donaldson, C.; Parkinson, A.; Schuchat, A.; Santosham, M.; Whitney, C. G. *J. Infect. Dis.* **2003,** *188*(1), 81-89.
[93] Breiman, R. F.; Keller, D. W.; Phelan, M. A.; Sniadack, D. H.; Stephens, D. S.; Rimland, D.; Farley, M. M.; Schuchat, A.; Reingold, A. L. *Arch. Inter. Med.* **2000,** *160*(17), 2633-2638.
[94] Sanders, L. A.; van-de-Winkel, J. G.; Rijkers, G. T.; Voorhorst-Ogink, M. M.; de-Haas, M.; Capel, P. J.; Zegers, B. J. *J. Infect. Dis.* **1994,** *170* 854-861.
[95] Abadi, J.; Zhong, Z.; Dobroszycki, J.; Pirofski, L. A. *Pediatr. Res.* **1997,** *42*(3), 259-262.
[96] Roy, S.; Hill, A. V.; Knox, K.; Griffiths, D.; Crook, D. *Br. Med. J.* **2002,** *324*(7350), 1369.
[97] Roy, S.; Knox, K.; Segal, S.; Griffiths, D.; Moore, C. E.; Welsh, K. I.; Smarason, A.; Day, N. P.; McPheat, W. L.; Crook, D. W.; Hill, A. V. *Lancet* **2002,** *359*(9317), 1569-1573.
[98] French, N. *J. Infect.* **2003,** *46*(2), 78-86.
[99] Austrian, R. *J. Infect. Dis.* **1977,** *136* Suppl, S38-S42.
[100] Simberkoff, M. S.; Cross, A. P.; Al-Ibrahim, M.; Baltch, A. L.; Geiseler, P. J.; Nadler, J.; Richmond, A. S.; Smith, R. P.; Schiffman, G.; Shepard, D. S.; *et al. N. Engl. J. Med.* **1986,** *315*(21), 1318-1327.
[101] Davis, A. L.; Aranda, C. P.; Schiffman, G.; Christianson, L. C. *Chest* **1987,** *92*(2), 204-212.
[102] Leech, J. A.; Gervais, A.; Ruben, F. L. *CMAJ* **1987,** *136*(4), 361-365.
[103] Koivula, I.; Sten, M.; Leinonen, M.; Makela, P. H. *Am. J. Med.* **1997,** *103*(4), 281-290.
[104] Ortqvist, A.; Hedlund, J.; Burman, L. A.; Elbel, E.; Hofer, M.; Leinonen, M.; Lindblad, I.; Sundelof, B.; Kalin, M. *Lancet* **1998,** *351*(9100), 399-403.
[105] Sims, R. V.; Steinmann, W. C.; McConville, J. H.; King, L. R.; Zwick, W. C.; Schwartz, J. S. *Ann. Intern. Med.* **1988,** *108*(5), 653-657.
[106] Shapiro, E. D.; Berg, A. T.; Austrian, R.; Schroeder, D.; Parcells, V.; Margolis, A.; Adair, R. K.; Clemens, J. D. *N. Engl. J. Med.* **1991,** *325*(21), 1453-1460.
[107] Farr, B. M.; Johnston, B. L.; Cobb, D. K.; Fisch, M. J.; Germanson, T. P.; Adal, K. A.; Anglim, A. M. *Arch. Intern. Med.* **1995,** *155*(21), 2336-2340.
[108] Gable, C. B.; Holzer, S. S.; Engelhart, L.; Friedman, R. B.; Smeltz, F.; Schroeder, D.; Baum, K. *JAMA* **1990,** *264*(22), 2910-2915.
[109] Sisk, J. E.; Moskowitz, A. J.; Whang, W.; Lin, J. D.; Fedson, D. S.; McBean, A. M.; Plouffe, J. F.; Cetron, M. S.; Butler, J. C. *JAMA* **1997,** *278*(16), 1333-1339.
[110] Black, S.; Shinefield, H.; Fireman, B. *Ped. Infect. Dis. J.* **2000,** *19* 187-195.
[111] Eskola, J.; Kilpi, T.; Palmu, A.; Jokinen, J.; Haapakoski, J.; Herva, E.; Takala, A.; Kayhty, H.; Karma, P.; Kohberger, R.; Siber, G.; Makela, P. H. *N. Engl. J. Med.* **2001,** *344*(6), 403-409.
[112] Kilpi, T.; Palmu, A.; Leinonen, M. Proceedings of 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, Tornonto, Canada September 2000; [40], 245.
[113] O'Brien, K. L.; Moulton, L. H.; Reid, R.; Weatherholtz, R.; Oski, J.; Brown, L.; Kumar, G.; Parkinson, A.; Hu, D.; Hackell, J.; Chang, I.; Kohberger, R.; Siber, G.; Santosham, M. *Lancet* **2003,** *362*(9381), 355-361.
[114] Veenhoven, R.; Bogaert, D.; Uiterwaal, C.; Brouwer, C.; Kiezebrink, H.; Bruin, J.; IJzerman, E.; Hermans, P.; de Groot, R.; Zegers, B.; Kuis, W.; Rijkers, G.; Schilder, A.; Sanders, E. *Lancet* **2003,** *361*(9376), 2189-2195.
[115] Klugman, K. P.; Madhi, S. A.; Huebner, R. E.; Kohberger, R.; Mbelle, N.; Pierce, N. *N. Engl. J. Med.* **2003,** *349*(14), 1341-1348.
[116] Choo, S.; Zhang, Q.; Seymour, L.; Akhtar, S.; Finn, A. *J. Infect. Dis.* **2000,** *182*(4), 1260-1263.

[117] Gordon, S. B.; Kanyanda, S.; Walsh, A. L.; Goddard, K.; Chaponda, M.; Atkinson, V.; Mulwafu, W.; Molyneux, E. M.; Zijlstra, E. E.; Molyneux, M. E.; Graham, S. M. *Emerg. Infect. Dis.* **2003,** *9*(6), 747-749.

[118] Reynolds, H. Y. *Curr. Opin. Pulmon. Med.* **2002,** *8*(3), 154-165.

[119] Weiser, J. N.; Markiewicz, Z.; Tuomanen, E. I.; Wani, J. H. *Infect. Immun.* **1996,** *64*(6), 2240-2245.

[120] Weiser, J. N.; Bae, D.; Epino, H.; Gordon, S. B.; Kapoor, M.; Zenewicz, L. A.; Shchepetov, M. *Infect. Immun.* **2001,** *69*(9), 5430-5439.

[121] Kim, J. O.; Weiser, J. N. *J. Infect. Dis.* **1998,** *177*(2), 368-377.

[122] Smith, T.; Lehmann, D.; Montgomery, J.; Gratten, M.; Riley, I. D.; Alpers, M. P. *Epidemiol. Infect.* **1993,** *111*(1), 27-39.

[123] Musher, D. M.; Groover, J. E.; Reichler, M. R.; Riedo, F. X.; Schwartz, B.; Watson, D. A.; Baughn, R. E.; Breiman, R. F. *Clin. Infect. Dis.* **1997,** *24*(3), 441-446.

[124] Lloyd-Evans, N.; O'Dempsey, T. J.; Baldeh, I.; Secka, O.; Demba, E.; Todd, J. E.; McArdle, T. F.; Banya, W. S.; Greenwood, B. M. *Ped. Infect. Dis. J.* **1996,** *15*(10), 866-871.

[125] Simell, B.; Kilpi, T. M.; Kayhty, H. *J. Infect. Dis.* **2002,** *186*(8), 1106-1114.

[126] Tuomanen, E. I.; Austrian, R.; Masure, H. R. *N. Engl. J. Med.* **1995,** *332*(19), 1280-1284.

[127] Cundell, D. R.; Gerard, N. P.; Gerard, C.; Idanpaan, H., I; Tuomanen, E. I. *Nature* **1995,** *377*(6548), 435-438.

[128] Ring, A.; Weiser, J. N.; Tuomanen, E. I. *J. Clin. Invest.* **1998,** *102*(2), 347-360.

[129] *Eur. Res. J.* **1992,** Vol. 5

[130] Lohmann, M. M.; Steinmuller, C.; Franke, U. G. *Eur. Respir. J.* **1994,** *7*(9), 1678-1689.

[131] Thepen, T.; van Rooijen, N.; Kraal, G. *J. Exp. Med.* **1989,** *170* 499-509.

[132] Thoma-Uszynski, S.; Stenger, S.; Takeuchi, O.; Ochoa, M. T.; Engele, M.; Sieling, P. A.; Barnes, P. F.; Rollinghoff, M.; Bolcskei, P. L.; Wagner, M.; Akira, S.; Norgard, M. V.; Belisle, J. T.; Godowski, P. J.; Bloom, B. R.; Modlin, R. L. *Science* **2001,** *291*(5508), 1544-1547.

[133] Murdoch, C.; Read, R. C.; Zhang, Q.; Finn, A. *J. Infect. Dis.* **2002,** *186*(9), 1253-1260.

[134] Doyle, N. A.; Bhagwan, S. D.; Meek, B. B.; Kutkoski, G. J.; Steeber, D. A.; Tedder, T. F.; Doerschuk, C. M. *J. Clin. Invest.* **1997,** *99*(3), 526-533.

[135] Dehoux, M. S.; Boutten, A.; Ostinelli, J.; Seta, N.; Dombret, M. C.; Crestani, B.; Deschenes, M.; Trouillet, J. L.; Aubier, M. *Am. J. Respir. Crit. Care Med.* **1994,** *150*(3), 710-716.

[136] Xia, W.; Pinto, C. E.; Kradin, R. L. *J. Exp. Med.* **1995,** *181*(4), 1275-1283.

[137] Holt, P. G. *Am. J. Respir. Crit. Care Med.* **2000,** *162*(4 Pt 2), S151-S156.

[138] Lambrecht, B. N.; Prins, J. B.; Hoogsteden, H. C. *Eur. Res. J.* **2001,** *18*(4), 692-704.

[139] Agostini, C.; Chilosi, M.; Zambello, R.; Trentin, L.; Semenzato, G. *Eur. Res. J.* **1993,** *6*(9), 1378-1401.

[140] Holt, P. G.; Oliver, J.; Bilyk, N.; McMenamin, C.; McMenamin, P. G.; Kraal, G.; Thepen, T. *J. Exp. Med.* **1993,** *177,* 397-407.

[141] Chelen, C. J.; Fang, Y.; Freeman, G. J.; Secrist, H.; Marshall, J. D.; Hwang, P. T.; Frankel, L. R.; DeKruyff, R. H.; Umetsu, D. T. *J. Clin. Invest.* **1995,** *95*(3), 1415-1421.

[142] Gordon, S. B.; Irving, G. R.; Lawson, R. A.; Lee, M. E.; Read, R. C. *Infect. Immun.* **2000,** *68*(4), 2286-2293.

[143] Watson, D. A.; Musher, D. M.; Verhoef, J. *Eur. J. Clin. Microbiol. Infect. Dis.* **1995,** *14*(6), 479-490.

[144] Tuomanen, E.; Liu, H.; Hengstler, B.; Zak, O.; Tomasz, A. *J. Infect. Dis.* **1985,** *151*(5), 859-868.

[145] Brown, E. J.; Hosea, S. W.; Frank, M. M. *Rev. Infect. Dis.* **1983,** *5*(Suppl. 4), S797-805.

[146] Ring, A.; Weiser, J. N.; Tuomanen, E. I. *J. Clin. Invest.* **1998,** *102*(2), 347-360.

[147] Lohmann-Matthes, M. L.; Steinmuller, C.; Franke-Ullmann, G. *Eur. Respir. J.* **1994,** *7*(9), 1678-1689.

[148] Blumenthal, R. L.; Campbell, D. E.; Hwang, P.; DeKruyff, R. H.; Frankel, L. R.; Umetsu, D. T. *J. Allergy Clin. Immunol.* **2001,** *107*(2), 258-264.

[149] Gong, J. L.; McCarthy, K. M.; Rogers, R. A.; Schneeberger, E. E. *Immunology* **1994,** *81*(3), 343-351.

[150] Ramirez, M. C.; Sigal, L. J. *J. Immunol.* **2002,** *169*(12), 6733-6742.

[151] Lipscomb, M. F.; Masten, B. J. *Physiol. Rev.* **2002,** *82* 97-130.

[152] Balmelli, C.; Demotz, S.; Acha-Orbea, H.; De Grandi, P.; Nardelli-Haefliger, D. *J. Virol.* **2002,** *76*(24), 12596-12602.

[153] Dieu, M. C.; Vanbervliet, B.; Vicari, A.; Bridon, J. M.; Oldham, E.; Ait-Yahia, S.; Briere, F.; Zlotnik, A.; Lebecque, S.; Caux, C. *J. Exp. Med.* **1998,** *188*(2), 373-386.

[154] Vermaelen, K. Y.; Carro-Muino, I.; Lambrecht, B. N.; Pauwels, R. A. *J. Exp. Med.* **2001,** *193*(1), 51-60.

[155] Power, C. A.; Church, D. J.; Meyer, A.; Alouani, S.; Proudfoot, A. E.; Clark-Lewis, I.; Sozzani, S.; Mantovani, A.; Wells, T. N. *J. Exp. Med.* **1997,** *186*(6), 825-835.

[156] Mosmann, T. R.; Cherwinski, H.; Bond, M. W.; Giedlin, M. A.; Coffman, R. L. *J. Immunol.* **1986,** *136*(7), 2348-2357.

[157] Stumbles, P. A.; Thomas, J. A.; Pimm, C. L.; Lee, P. T.; Venaille, T. J.; Proksch, S.; Holt, P. G. *J. Exp. Med.* **1998,** *188*(11), 2019-2031.

[158] Edwards, A. D.; Manickasingham, S. P.; Sporri, R.; Diebold, S. S.; Schulz, O.; Sher, A.; Kaisho, T.; Akira, S.; Reis e Sousa *J. Immunol.* **2002,** *169*(7), 3652-3660.

[159] Trinchieri, G. *Nat. Rev. Immunol.* **2003,** *3*(2), 133-146.

[160] Kikuchi, T.; Crystal, R. G. *J. Clin. Invest* **2001,** *108*(6), 917-927.

[161] Kanayama, N.; Kimoto, T.; Todo, K.; Nishikawa, Y.; Hikida, M.; Magari, M.; Cascalho, M.; Ohmori, H. *J. Immunol.* **2002,** *169*(12), 6865-6874.

[162] Niiro, H.; Clark, E. A. *Nat. Rev. Immunol.* **2002,** *2* 945-956.

[163] D'Ambrosio, D.; Mariani, M.; Panina-Bordignon, P.; Sinigaglia, F. *Am. J. Respir. Crit Care Med.* **2001,** *164*(7), 1266-1275.

[164] Sabroe, I.; Lloyd, C. M.; Whyte, M. K.; Dower, S. K.; Williams, T. J.; Pease, J. E. *Eur. Respir. J.* **2002,** *19*(2), 350-355.

[165] Kopf, M.; Abel, B.; Gallimore, A.; Carroll, M.; Bachmann, M. F. *Nat. Med.* **2002,** *8*(4), 373-378.

[166] Bluestone, J. A.; Abbas, A. K. *Nat. Rev. Immunol.* **2003,** *3* 253-257.

[167] Out, T. A.; Wang, S. Z.; Rudolph, K.; Bice, D. E. *Immunology* **2002,** *105*(4), 499-508.

[168] Hoyne, G. F.; Tan, K.; Corsin-Jimenez, M.; Wahl, K.; Stewart, M.; Howie, S. E.; Lamb, J. R. *Am. J. Respir. Crit. Care Med.* **2000,** *162*(4 Pt 2), S169-S174.

[169] Neurath, M. F.; Finotto, S.; Glimcher, L. H. *Nat. Med.* **2002,** *8*(6), 567-573.

[170] Garrone, P.; Neidhardt, E.-M.; Garcia, E.; Galibert, L.; van Kooten, C.; Banchereau, J. *J. Exp. Med.* **1995,** *182* 1265-1273.

[171] Scott, D. W.; Grdina, T.; Shi, Y. *J. Immunol.* **1996,** *156* 2352-2356.

[172] Kehrl, J. H.; Fauci, A. S. *J. Clin. Invest.* **1982,** *71* 1032-1040.

[173] Vinuesa, C.G.; MacLennan, I.C. In *Immunology of Carbohydrates*, Wong, S. Y.; Arsequell, G., editors; Kluwer Academic/Plenum Publishers: **2003**; pp. 128-147.

[174] Rijkers, G. T.; Mosier, D. E. *J. Immunol.* **1985,** *135*(1), 1-4.

[175] Baker, P. J.; Amsbaugh, D. F.; Stashak, P. W.; Caldes, G.; Prescott, B. *Rev. Infect. Dis.* **1981,** *3*(2), 332-341.

[176] Stäb, F.; Austrup, F.; issi, E.; Kölsch, E. *J. Immunol.* **1990,** *144* 53-59.

[177] Dullforce, P.; Sutton, D. C.; Heath, A. W. *Nature Med.* **1998,** *4*(1), 88-91.

[178] Bondada, S.; Wu, H.; Robertson, D. A.; Chelvarajan, R. L. *Vaccine* **2000,** *19*(4-5), 557-565.

[179] Buchanan, R. M.; Arulanandam, B. P.; Metzger, D. W. *J. Immunol.* **1998,** *161*(10), 5525-5533.

[180] Colino, J.; Shen, Y.; Snapper, C. M. *J. Exp. Med.* **2002,** *195*(1), 1-13.

[181] Zandvoort, A.; Timens, W. *Clin. Exp. Immunol.* **2002,** *130*(1), 4-11.

[182] Garg, M.; Kaplan, A. M.; Bondada, S. *J. Immunol.* **1994,** *152*(4), 1589-1596.

[183] Martin, F.; Kearney, J. F. *Immunol. Rev.* **2000,** *175,* 70-79.

[184] Wilkes, D. S.; Weissler, J. C. In *Lung macrophages and dendritic cells in health and disease*, 1 ed.; Lipscomb, M. F.; Russell, S. W., editors; Marcel Dekker: New York, **1997**; Vol. *102,* Chapter 14, pp. 335-369.

[185] Harding, C. V.; Kihlberg, J.; Elofsson, M.; Magnusson, G.; Unanue, E. R. *J. Immunol.* **1993,** *151* 2419-2425.

[186] Feikin, D. R.; Elie, C. M.; Goetz, M. B.; Lennox, J. L.; Carlone, G. M.; Romero-Steiner, S.; Holder, P. F.; O'Brien, W. A.; Whitney, C. G.; Butler, J. C.; Breiman, R. F. *Vaccine* **2001,** *20*(3-4), 545-553.

[187] Goldblatt, D.; Borrow, R.; Miller, E. *J. Infect. Dis.* **2002,** *185*(3), 397-400.

[188] Arva, E.; Dahlgren, U.; Lock, R.; Andersson, B. *Int. Arch. Allergy Immunol.* **1996,** *109*(1), 35-43.
[189] Wu, H. Y.; Nahm, M. H.; Guo, Y.; Russell, M. W.; Briles, D. E. *J. Infect. Dis.* **1997,** *175*(4), 839-846.
[190] Yamamoto, M.; Briles, D. E.; Yamamoto, S.; Ohmura, M.; Kiyono, H.; McGhee, J. R. *J. Immunol.* **1998,** *161*(8), 4115-4121.
[191] Jakobsen, H.; Schulz, D.; Pizza, M.; Rappuoli, R.; Jonsdottir, I. *Infect. Immun.* **1999,** *67*(11), 5892-5897.
[192] Arulanandam, B. P.; O'Toole, M.; Metzger, D. W. *J. Infect. Dis.* **1999,** *180*(4), 940-949.
[193] McCool, T. L.; Cate, T. R.; Moy, G.; Weiser, J. N. *J. Exp. Med.* **2002,** *195*(3), 359-365.
[194] McCool, T. L.; Cate, T. R.; Tuomanen, E. I.; Adrian, P.; Mitchell, T. J.; Weiser, J. N. *Infect. Immun.* **2003,** *71*(10), 5724-5732.
[195] Bice, D. E.; Jones, S. E.; Muggenburg, B. A. *Am. J. Respir. Cell Mol. Biol.* **1993,** *8*(6), 662-667.
[196] Davis, S. S. *Adv. Drug Deliv. Rev.* **2001,** *51*(1-3), 21-42.
[197] Hagiwara, Y.; McGhee, J. R.; Fujihashi, K.; Kobayashi, R.; Yoshino, N.; Kataoka, K.; Etani, Y.; Kweon, M. N.; Tamura, S.; Kurata, T.; Takeda, Y.; Kiyono, H.; Fujihashi, K. *J. Immunol.* **2003,** *170*(4), 1754-1762.
[198] Michon, F.; Fusco, P. C.; Minetti, C. A.; Laude-Sharp, M.; Uitz, C.; Huang, C. H.; D'Ambra, A. J.; Moore, S.; Remeta, D. P.; Heron, I.; Blake, M. S. *Vaccine* **1998,** *16*(18), 1732-1741.
[199] Ogunniyi, A. D.; Folland, R. L.; Briles, D. E.; Hollingshead, S. K.; Paton, J. C. *Infect. Immun.* **2000,** *68*(5), 3028-3033.
[200] Ugozzoli, M.; Mariani, M.; Del Giudice, G.; Soenawan, E.; O'Hagan, D. T. *J. Infect. Dis.* **2002,** *186*(9), 1358-1361.
[201] Levine, M. M. *Nat. Med.* **2003,** *9*(1), 99-103.
[202] Lehner, T.; Anton, P. A. *AIDS* **2002,** *16* (Suppl. 4) S125-S132.
[203] McCluskie, M. J.; Davis, H. L. *J. Immunol.* **1998,** *161*(9), 4463-4466.
[204] Doherty, T. M.; Olsen, A. W.; van Pinxteren, L.; Andersen, P. *Infect. Immun.* **2002,** *70*(6), 3111-3121.
[205] Jiao, X.; Hirano, T.; Hou, Y.; Gu, X. X. *Infect. Immun.* **2002,** *70*(11), 5982-5989.
[206] George-Chandy, A.; Mielcarek, N.; Nordstrom, I.; Holmgren, J.; Eriksson, K. *Infect. Immun.* **2001,** *69*(6), 4120-4124.
[207] Strickland, D. H.; Kees, U. R.; Holt, P. G. *Eur. Res. J.* **1994,** *7*(12), 2124-2130.
[208] Fujihashi, K.; Koga, T.; van Ginkel, F. W.; Hagiwara, Y.; McGhee, J. R. *Vaccine* **2002,** *20*(19-20), 2431-2438.
[209] Zysk, G.; Bongaerts, R. J.; ten Thoren, E.; Bethe, G.; Hakenbeck, R.; Heinz, H. P. *Infect. Immun.* **2000,** *68*(6), 3740-3743.
[210] Casal, J.; Tarrago, D. *Curr. Opin. Infect. Dis.* **2003,** *16*(3), 219-224.
[211] Jagger, M. P.; Huo, Z.; Riches, P. G. *Clin. Exp. Immunol.* **2002,** *130*(3), 467-474.
[212] Cabellos, C.; MacIntyre, D. E.; Forrest, M.; Burroughs, M.; Prasad, S.; Tuomanen, E. *J. Clin. Invest.* **1992,** *90*(2), 612-618.
[213] Alexander, J. E.; Lock, R. A.; Peeters, C. C.; Poolman, J. T.; Andrew, P. W.; Mitchell, T. J.; Hansman, D.; Paton, J. C. *Infect. Immun.* **1994,** *62*(12), 5683-5688.
[214] Briles, D. E.; Yother, J.; McDaniel, L. S. *Rev. Infect. Dis.* **1988,** *10* (Suppl. 2), S372-S374.
[215] Crain, M. J.; Waltman, W. D.; Turner, J. S.; Yother, J.; Talkington, D. F.; McDaniel, L. S.; Gray, B. M.; Briles, D. E. *Infect. Immun.* **1990,** *58*(10), 3293-3299.
[216] McDaniel, L. S.; Scott, G.; Widenhofer, K.; Carroll, J. M.; Briles, D. E. *Mic. Pathog.* **1986,** *1*(6), 519-531.
[217] Talkington, D. F.; Crimmins, D. L.; Voellinger, D. C.; Yother, J.; Briles, D. E. *Infect. Immun.* **1991,** *59*(4), 1285-1289.
[218] Yother, J.; Handsome, G. L.; Briles, D. E. *J. Bacteriol.* **1992,** *174*(2), 610-618.
[219] Lamani, E.; McPherson, D. T.; Hollingshead, S. K.; Jedrzejas, M. J. *Prot. Exp. Purif.* **2000,** *20*(3), 379-388.
[220] Nabors, G. S.; Braun, P. A.; Herrmann, D. J.; Heise, M. L.; Pyle, D. J.; Gravenstein, S.; Schilling, M.; Ferguson, L. M.; Hollingshead, S. K.; Briles, D. E.; Becker, R. S. *Vaccine* **2000,** *18*(17), 1743-1754.
[221] Yamamoto, M.; McDaniel, L. S.; Kawabata, K.; Briles, D. E.; Jackson, R. J.; McGhee, J. R.; Kiyono, H. *Infect. Immun.* **1997,** *65*(2), 640-644.
[222] Briles, D. E.; Hollingshead, S. K.; Paton, J. C.; Ades, E. W.; Novak, L.; van Ginkel, F. W.; Benjamin, W. H., Jr. *J. Infect. Dis.* **2003,** *188*(3), 339-348.
[223] Arulanandam, B. P.; Lynch, J. M.; Briles, D. E.; Hollingshead, S.; Metzger, D. W. *Infect. Immun.* **2001,** *69*(11), 6718-6724.
[224] Zysk, G.; Bethe, G.; Nau, R.; Koch, D.; Grafin, V. B., V; Heinz, H. P.; Reinert, R. R. *J. Infect. Dis.* **2003,** *187*(2), 330-333.
[225] Simell, B.; Korkeila, M.; Pursiainen, H.; Kilpi, T. M.; Kayhty, H. *J. Infect. Dis.* **2001,** *183*(6), 887-896.
[226] Virolainen, A.; Russell, W.; Crain, M. J.; Rapola, S.; Kayhty, H.; Briles, D. E. *Pediatr. Infect. Dis. J.* **2000,** *19*(2), 134-138.
[227] Rapola, S.; Jantti, V.; Haikala, R.; Syrjanen, R.; Carlone, G. M.; Sampson, J. S.; Briles, D. E.; Paton, J. C.; Takala, A. K.; Kilpi, T. M.; Kayhty, H. *J. Infect. Dis.* **2000,** *182*(4), 1146-1152.
[228] Briles, D. E.; Hollingshead, S. K.; King, J.; Swift, A.; Braun, P. A.; Park, M. K.; Ferguson, L. M.; Nahm, M. H.; Nabors, G. S. *J. Infect. Dis.* **2000,** *182*(6), 1694-1701.
[229] Berry, A. M.; Paton, J. C. *Infect. Immun.* **1996,** *64*(12), 5255-5262.
[230] Morrison, K. E.; Lake, D.; Crook, J.; Carlone, G. M.; Ades, E.; Facklam, R.; Sampson, J. S. *J. Clin. Microbiol.* **2000,** *38*(1), 434-437.
[231] De, B. K.; Woolfitt, A. R.; Barr, J. R.; Daneshvar, M. I.; Sampson, J. S.; Ades, E. W.; Carlone, G. M. *Arch. Biochem. Biophy.* **2003,** *419*(2), 147-157.
[232] Gor, D. O.; Ding, X.; Li, Q.; Schreiber, J. R.; Dubinsky, M.; Greenspan, N. S. *Infect. Immun.* **2002,** *70*(10), 5589-5595.
[233] Scott, J. A.; Obiero, J.; Hall, A. J.; Marsh, K. *J. Infect. Dis.* **2002,** *186*(2), 220-226.
[234] Rapola, S.; Jantti, V.; Eerola, M.; Makela, P. H.; Kayhty, H.; Kilpi, T. *Vaccine* **2003,** *21*(25-26), 3608-3613.
[235] Zhang, Q.; Choo, S.; Finn, A. *Infect. Immun.* **2002,** *70*(10), 5363-5369.
[236] Johnson, S. E.; Dykes, J. K.; Jue, D. L.; Sampson, J. S.; Carlone, G. M.; Ades, E. W. *J. Infect. Dis.* **2002,** *185*(4), 489-496.
[237] Talkington, D. F.; Brown, B. G.; Tharpe, J. A.; Koenig, A.; Russell, H. *Microb. Pathog.* **1996,** *21*(1), 17-22.
[238] Miyaji, E. N.; Dias, W. O.; Gamberini, M.; Gebara, V. C.; Schenkman, R. P.; Wild, J.; Riedl, P.; Reimann, J.; Schirmbeck, R.; Leite, L. C. *Vaccine* **2001,** *20*(5-6), 805-812.
[239] Seo, J. Y.; Seong, S. Y.; Ahn, B. Y.; Kwon, I. C.; Chung, H.; Jeong, S. Y. *Infect. Immun.* **2002,** *70*(3), 1143-1149.
[240] Lock, R. A.; Hansman, D.; Paton, J. C. *Microb. Pathog.* **1992,** *12*(2), 137-143.
[241] Lankinen, K. S.; Ruutu, P.; Nohynek, H.; Lucero, M.; Paton, J. C.; Leinonen, M. *Scand. J. Infect. Dis.* **1999,** *31*(2), 155-161.
[242] Rapola, S.; Kilpi, T.; Lahdenkari, M.; Makela, P. H.; Kayhty, H. *Pediatr. Infect. Dis. J.* **2001,** *20*(5), 482-487.
[243] Ogunniyi, A. D.; Woodrow, M. C.; Poolman, J. T.; Paton, J. C. *Infect. Immun.* **2001,** *69*(10), 5997-6003.
[244] Yamamoto, M.; McGhee, J. R.; Hagiwara, Y.; Otake, S.; Kiyono, H. *Scand. J. Immunol.* **2001,** *53*(3), 211-217.
[245] Balachandran, P.; Brooks-Walter, A.; Virolainen-Julkunen, A.; Hollingshead, S. K.; Briles, D. E. *Infect. Immun.* **2002,** *70*(5), 2526-2534.
[246] Seo, J. Y.; Seong, S. Y.; Ahn, B. Y.; Kwon, I. C.; Chung, H.; Jeong, S. Y. *Infect. Immun.* **2002,** *70*(3), 1143-1149.
[247] Bice, D. E.; Muggenburg, B. A. *Immunol.* **1996,** *88*(2), 191-197.
[248] van Eeden, S. F.; Tan, W. C.; Suwa, T.; Mukae, H.; Terashima, T.; Fujii, T.; Qui, D.; Vincent, R.; Hogg, J. C. *Am. J. Respir. Crit. Care Med.* **2001,** *164*(5), 826-830.
[249] Ghio, A. J.; Devlin, R. B. *Am. J. Respir. Crit. Care Med.* **2001,** *164*(4), 704-708.
[250] Becker, S.; Fenton, M. J.; Soukup, J. M. *Am. J. Resp. Cell Mol. Biol.* **2002,** *27*(5), 611-618.
[251] Ezzati, M.; Kammen, D. *Lancet* **2001,** *358*(9282), 619-624.
[252] Smith, K. R.; Samet, J. M.; Romieu, I.; Bruce, N. *Thorax* **2000,** *55*(6), 518-532.
[253] Gordon, S. B.; Curran, A. D.; Turley, A.; Wong, C. H.; Rahman, S. N.; Wiley, K.; Morice, A. H. *Amer. J. Respir. Crit. Care Med.* **1997,** *156*(1), 206-210.
[254] Morice, A. H.; Kastelik, J. A.; Thompson, R. *Br. J. Clin. Pharmacol.* **2001,** *52*(4), 365-375.
[255] Valdez, H.; Mitsuyasu, R.; Landay, A.; Sevin, A. D.; Chan, E. S.; Spritzler, J.; Kalams, S. A.; Pollard, R. B.; Fahey, J.; Fox, L.; Namkung, A.; Estep, S.; Moss, R.; Sahner, D.; Lederman, M. M. *J. Infect. Dis.* **2003,** *187*(2), 320-325.
[256] Jacobson, M. A.; Spritzler, J.; Landay, A.; Chan, E.; Katzenstein, D.; Schock, B.; Fox, L.; Roe, J.; Kundu, S.; Pollard, R. *AIDS* **2002,** *16*(8), 1147-1154.

[257] Steinhauser, M. L.; Hogaboam, C. M.; Kunkel, S. L.; Lukacs, N. W.; Strieter, R. M.; Standiford, T. J. *J. Immunol.* **1999,** *162*(1), 392-399.

[258] Qureshi, M. H.; Harmsen, A. G.; Garvy, B. A. *J. Immunol.* **2003,** *170*(2), 1002-1009.

[259] Opal, S. M.; Keith, J. C., Jr.; Jhung, J.; Palardy, J. E.; Parejo, N.; Marchese, E.; Maganti, V. *J. Infect. Dis.* **2003,** *187*(1), 70-76.

[260] Lauw, F. N.; Branger, J.; Florquin, S.; Speelman, P.; Van Deventer, S. J.; Akira, S.; Van Der, P. T. *J. Immunol.* **2002,** *168*(1), 372-378.

[261] Chen, G. H.; Reddy, R. C.; Newstead, M. W.; Tateda, K.; Kyasapura, B. L.; Standiford, T. J. *J. Immunol.* **2000,** *165*(11), 6496-6503.

[262] Wu, M.; Hussain, S.; He, Y. H.; Pasula, R.; Smith, P. A.; Martin, W. J. *Proc. Natl. Acad. Sci. U.S.A* **2001,** *98*(25), 14589-14594.

[263] Flohe, S. B.; Bruggemann, J.; Lendemans, S.; Nikulina, M.; Meierhoff, G.; Flohe, S.; Kolb, H. *J. Immunol.* **2003,** *170*(5), 2340-2348.

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