

Expert Opinion

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Pneumococcal vaccines for allergic airways diseases

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Background: Asthma is a common global health problem. Environmental exposures such as bacteria may protect against asthma development. **Objective:** This review aims to examine the possible protective role of pneumococcal infection and vaccination in asthma. **Methods:** A review of known experimental biology and human epidemiology relating to asthma and pneumococcal infection was performed. **Results:** Pneumococcal infection can modulate components of allergic airways disease such as airways hyper-responsiveness and airway eosinophilia. Exposure to killed pneumococcus can reproduce these effects and the mechanism may involve control by T regulatory cells. **Conclusions:** Pneumococcal immunoregulatory therapy is a potentially important approach to asthma management that requires further evaluation in well-designed research studies.

Keywords: allergy, asthma, eosinophil, immunoregulatory, infection, *Streptococcus pneumoniae*, T regulatory cell, vaccine

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1. Introduction

Asthma is a common illness throughout the world, with a significant geographic variation in prevalence. In Australia and parts of Europe and North America the prevalence is high [1], with rates of current asthma affecting up to 1 in 10 adults and 1 in 7 children. This contrasts with some African countries where the prevalence can be as low as 2% [1]. There has also been a dramatic rise in the prevalence of asthma. While the most recent data indicate a stabilization of asthma prevalence in the countries with the highest rates, asthma still appears to be increasing in countries with intermediate asthma rates. A study using objective confirmation of asthma, that was independent of physician's diagnosis, found there had been at least a doubling of asthma prevalence in school age children in Eastern Australia between 1980 and 1990 [2], that has subsequently stabilized [3]. Migration studies also demonstrate that children can develop asthma when they move from a low-prevalence country to a country with high asthma prevalence. Adolescents who migrated to Australia from countries with low asthma prevalence experienced an 11% increase in the prevalence of wheezing for every year of residence in Australia [4]. These observations demonstrate that there are significant environmental influences that affect asthma, and indicate a pressing need to understand these changes and develop interventions that will reduce the burden of illness from asthma.

The mechanisms underlying asthma involve immune-mediated airway inflammation, with evidence of both genetic and environmental modulation of asthmatic responses. Genetic factors confer susceptibility, and interact with environmental exposures to permit expression of the disease. The recent world-wide changes in asthma prevalence imply significant environmental effects, and also hold out the possibility that modification of exposures or the subsequent immune responses, termed immunoregulatory therapy, may reduce the burden of asthma. A key

exposure that may be related to the development of asthma is exposure to microbial species. Exposure to certain bacteria may either increase or reduce asthma risk, and we have proposed that infection with the respiratory pathogen *Streptococcus pneumoniae* may be protective against asthma. This review will examine the evidence for this proposal from human epidemiological studies and from intervention studies in model systems.

2. Exposure to infectious agents and asthma risk

Epidemiological studies have revealed that the increase in the prevalence of asthma has been associated with a reduction in exposure to infectious agents throughout life. For example, Jones *et al.* [5] collated data to show that those countries with the highest risk of childhood bacterial infection had the lowest risk of asthma. Specifically, there was a high ratio of deaths from asthma compared with those from tuberculosis in countries with a high asthma prevalence, whereas countries with a low asthma prevalence had a low ratio [5].

Further support for the association between exposure to infectious agents and asthma risk is provided by studies of antibiotic use and asthma. Infants who are given antibiotics in the first year of life have a greater risk of subsequently developing childhood wheezing [6,7]. The mechanisms of this association may involve modulation of intestinal microflora by antibiotic use [8], and studies are now trying to establish if the association is a causal relationship or a confounding effect [9].

Other epidemiological studies have shown a relationship between tuberculin reactivity in populations and reduced asthma risk [10], and between neonatal BCG vaccination and reduced asthma risk [11]. This has led to studies showing that immunomodulatory therapy with Bacille Calmette-Guerin (BCG) in animal model systems leads to reductions in allergic responses. The efficacy of mycobacterial immunization in preventing allergy in human trials has been varied, with decreases in the severity of atopic dermatitis but no effect on allergic asthma. Successful results were also dependent on the age of study subjects [12-17]. Exposure to bacterial DNA sequences, termed CpG oligodeoxynucleotides (CpG-ODN) can also modulate inflammatory responses. CpG stimulation of lymphocytes results in the development of potent T-helper-1 (Th1)-biased immune responses that have proven effective in suppressing allergic inflammation in mouse models [18]. These observations are now being tested in preclinical studies.

These studies demonstrate that there may be specificity in the protective antibacterial responses. The determinants of this specificity are not known but potentially involve the bacterial species used, the dose of the active moieties (e.g., endotoxin) and the pattern-recognition receptors that are engaged by the agent used. The studies also show that there is a need to be cautious in the interpretation of results

since translation of successful results in model systems may not be replicated in human trials [19], and also, unwanted adverse inflammatory effects may result from immune deviation. For example, neutrophilic airway response has been observed with high doses of CpG, which is consistent with the induction of Th1 responses by CpG [20]. The potential efficacy of pneumococcal vaccination for asthma needs to be considered against this background.

3. Spn and asthma risk

Streptococcus pneumoniae (Spn) is a common respiratory pathogen, and is the predominant cause of community-acquired pneumonia in children and adults. It also frequently causes otitis media, septicemia and meningitis. Invasive Spn infections are the sixth leading cause of death worldwide. Spn infection has not previously been widely implicated in the development and exacerbation of asthma. One recent study suggested that neonatal (first month of life only) exposure to commensal Spn was associated with a increased asthma risk [21]. Asthma has also been observed as an independent risk factor for invasive Spn disease [22-24]. Talbot *et al.* [22] conducted a nested case control study of 635 people with invasive pneumococcal disease and 6350 controls. Asthma was significantly overrepresented in the cases with invasive pneumococcal disease, such that people with asthma were found to have a 2.4-fold increased odds of having invasive pneumococcal disease compared with people without asthma. The risk was further increased in severe asthma, where there was an almost fourfold increase in the annual incidence of invasive pneumococcal disease compared with controls without asthma. A serotype analysis of the invasive pneumococcal isolates revealed that 56% of isolates were covered by the polyvalent conjugate vaccine, and 86% were covered by the 23-valent polysaccharide vaccine. Twenty-nine percent of isolates were covered only by the polysaccharide vaccine. This study raises important questions about the relationship between asthma and Spn and highlights the need for further studies of mechanisms and treatment of Spn in asthma.

4. Spn vaccination and asthma

Studies of Spn vaccination that include asthmatics also provide an opportunity to examine the interaction between Spn and asthma. These studies are few in number since asthmatics are seldom recommended as a target group in vaccination schedules using pneumococcal vaccines. Vaccination of elderly patients with a pneumococcal polysaccharide vaccine was associated with a reduction in the number and severity of hospitalizations for asthma in a retrospective study [25]. The vaccine used in this study was the 23-valent polysaccharide vaccine. A Cochrane systematic review [26] examined the effect of pneumococcal vaccination in asthma and identified three potential studies. One study [27] was a randomized controlled trial that examined the effect of pneumococcal

vaccination (and several other interventions) in children with asthma and recurrent (over four episodes in a year) otitis media. The study reported that the children treated with pneumococcal vaccination had a 30% reduction in asthma episodes over a 2-year period.

5. Spn and eosinophil responses

The inflammatory process in asthma that is induced by allergen exposure typically involves increases in eosinophils in several body compartments, including the airway, circulation and bone marrow. Eosinophilia is typical of allergic asthma. Experimental studies of eosinophil responses have observed that Spn infection can suppress circulating eosinophil counts [28-31]. When a pneumococcal abscess was induced in an experimental model of parasitic eosinophilia due to trichinosis, there was a rapid fall in circulating eosinophils that was unrelated to adrenal activity.

6. Phases of asthma

The potential for immunoregulatory intervention in asthma can be classified into three distinct phases: phase 1 intervention occurs prior to the development of allergic sensitization; phase 2 intervention occurs after sensitization and before the development of clinical asthma; and phase 3 intervention is given during active asthma, for example, during an asthma exacerbation.

Immunoregulatory therapy for the first phase corresponds to primary prevention of asthma and is designed to reduce sensitization to allergens that provoke asthma. Sensitization involves an interaction between innate and acquired immune responses. When a susceptible host is exposed to an allergen, the allergen is processed and presented to the immune active cells (typically T helper cells) by antigen presenting cells, such as dendritic cells, and there is a subsequent expansion of allergen specific T lymphocytes that exhibit a T-helper-2 (Th2) cytokine secretion profile, and generation of allergen-specific IgE by activated/matured B cells [32]. Sensitization to the allergens associated with asthma usually occurs in infancy and childhood. Sensitization to ingested allergens occurs during infancy, and aeroallergens induce sensitization in mid to late childhood. Immunoregulatory therapy to prevent sensitization would need to be administered in early childhood in order to be successful, and represents an example of primary disease prevention. There are several challenges to this approach, including the limited responsiveness to vaccines during infancy.

Once sensitized, there is a latency period before the clinical expression of asthma. During this period a person has evidence of allergen-specific IgE but does not have symptoms of asthma. This is termed the atopic state, and in western and developed countries up to 40% of the population are sensitized (atopic) to aeroallergens. This is an attractive target period for immunoregulatory therapy since subjects can be

identified by screening for allergen specific IgE and are generally in a vaccine-responsive period of life [33]. Intervention during this phase may be considered secondary prevention of asthma.

The third period for consideration of immunoregulatory therapy is during active asthma. As described above, up to 1 in 10 adults and 1 in 7 children have current asthma. Most children (>80%) and many adults (60%) have current asthma occurring in association with atopic sensitization. These people present to doctors for care and management strategies aimed at improving current clinical status and preventing future disease related events, a phase termed tertiary prevention [34].

7. Immunopathology of asthma (and allergic sensitization)

The immunopathology of asthma is characterised by the infiltration of eosinophils and T-lymphocytes into the airway, which is associated with increased mucus production, vascular exudation, and epithelial injury and epithelial desquamation. There is in addition a series of changes in airway structural elements such as smooth muscle that involve hypertrophy, hyperplasia and infiltration of mast cells into the muscle. The inflammatory response can be heterogeneous, with some patients demonstrating a relatively eosinophil-poor inflammatory pattern that is more commonly seen in severe adult asthma. The production of the Th2 cytokines IL-4, IL-5 and IL-13 by activated CD4⁺ T-lymphocytes is instrumental in disease pathogenesis in allergic asthma and mediates features of pathophysiology including IgE production, eosinophilia, mucus hypersecretion and airway hyperresponsiveness (AHR). While asthma is mainly Th2 driven, responses involving Th1 cells and IFN- γ are induced in severe asthmatics [35]. The mechanisms of how IFN- γ contributes to the immunopathology of asthma are unclear, but may involve the induction of apoptosis of bronchial epithelial cells [36].

8. T regulatory cells and asthma pathogenesis

Several independent studies have linked impaired or altered T regulatory cells (Tregs) in patients with asthma compared to healthy individuals [37-45]. Tregs have the ability to suppress effector responses involved in the pathogenesis of asthma and therefore have been considered as potential therapeutic targets for upregulation. IL-10 and TGF- β have been highlighted as important mediators of suppression. Indeed, corticosteroids prevent the protective effects of Tregs by inhibiting IL-10 [46] and membrane-bound TGF- β is a candidate for predicting the severity of asthma [47]. Adoptive transfer of CD4⁺CD25⁺ Tregs in animal models of allergic airways disease (AAD) has reinforced their potential as a therapeutic agent [48]. Studies in allergic patients treated with immunotherapy inducing Tregs have been associated with suppression of allergy [49,50]. Therefore the administration

of a targeted immunotherapy that reconstitutes the immune response through induction of Tregs is an attractive therapeutic for suppression of asthma.

9. Immune responses to Spn

Spn induces innate as well as adaptive (antibody and T-cell) immune responses.

Spn contains numerous pathogen-associated molecular patterns that activate toll-like receptors (TLRs), TLR-2, TLR-4 and the adaptor protein myeloid differentiation primary response gene 88 (MyD88) on innate cells including neutrophils and macrophages [51-54]. These phagocytic cells enter the infected area and are effective at phagocytosing and destroying the bacteria but these responses may also result in the suppression of Th2 responses and antagonize eosinophil influx in AADs.

It has been known for many years that anti-capsular antibody production is sufficient to protect against- and clear Spn infections [55,56]. However, help from T cells is necessary for the production and maturation of antibodies as well as class switching and the induction of memory of B cells [57,58]. T cell memory is also induced by infection, however the nature of the T cell response induced by infection is controversial and is dependent on the strain of Spn and the genetic background of the infected individual. Recently it has been shown that IL-17A is released from T cells upon infection. The activities of this cytokine include neutrophil activation and chemotaxis in concert with TLR-mediated IL-23 release from dendritic cells (DCs) [59]. This reinforces the neutrophil influx induced by innate responses and may further contribute to the suppression of AADs. Importantly, unlike other Gram-positive bacteria, Spn infection also induces the production of Tregs [60]. These Tregs may be important in protection against infection by controlling inflammatory responses. A consequent of the production of these Tregs may be the attenuation of Th2 responses in asthma [61].

Therefore, both innate and adaptive immune responses are induced by Spn infection that may suppress Th2 responses and AADs and asthma by multiple different mechanisms.

Although Spn infection and vaccine development are a clear research focus and investigated world wide, knowledge of the mechanisms underlying immunoregulation is in its infancy. Detailed elucidation of the mechanisms involved is the focus of in-depth studies in our laboratories.

10. Models to test the hypothesis

The assessment of Spn immunoregulatory therapy requires initial evaluation in model systems. This involves development of a model of allergic sensitization, a model of non-lethal Spn infection, and then a combined allergen-Spn model. There are several murine models that can be used to investigate different features of asthma, however, no single model

accurately represents all of the features of human disease. These models use an antigen, often ovalbumin, with a Th2-inducing adjuvant to induce sensitization and allergic responses. Other antigens such as cockroach extract or house dust mites may also be used, which may not require the co-administration of adjuvants [62,63]. Models have been developed using variations in mouse strain, and modifications of timing, route, dose and method of antigen administration. These effects are assessed using a variety of methods to examine tissue inflammatory and repair responses as well as accompanying physiological changes.

The closest approximations of chronic asthma and acute exacerbations of chronic asthma use long-term low dose aerosol challenge with ovalbumin [64,65]. Chronic models have characteristics of chronic inflammation accompanied by the recruitment of intra-epithelial eosinophils as well as features of airway remodelling similar to that observed in asthmatics including sub-epithelial fibrosis, epithelial thickening and mucus cell hyperplasia/metaplasia. This model also exhibits characteristic physiological changes of AHR to inhaled bronchoconstrictors. A single moderate-level antigen challenge induces an exacerbation that is characterised by an acute inflammatory component in the distal (intrapulmonary) airways with enhanced recruitment of CD3⁺ T cells, as well as exaggerated AHR. This can be superimposed on the chronic asthma model in order to mimic the *in vivo* situation of acute on chronic allergen exposure and subsequent responses.

Use of chronic models takes a long time and is labour-intensive and, therefore, other acute models are used extensively to model acute phases of disease [66,67]. Typically an antigen such as ovalbumin is administered systemically, which induces the development of potent Th2 responses. Adaptive immune responses to this sensitization are allowed to mature before rechallenge with cognate antigen occurs. This attracts memory Th2 cells into the lung, which release Th2 cytokines including IL-4, -5 and -13 that, in turn, promote the influx of eosinophils into the airways and the development of mucus secreting hyperplasia and metaplasia. These are models of acute broncho-pulmonary inflammation [67] and allergic inflammatory responses that together induce the development of AHR.

11. Non-lethal Spn models

Most mouse models of Spn infection are lethal models, which are used to test the efficacy of vaccines and treatments on serious invasive Spn disease. In order to investigate the effects of Spn infection on asthma using mouse models, non-lethal models of Spn infections are required. These have been developed using different strains of mice and use either lower doses of highly pathogenic strains [68] or less virulent strains [69]. These non-lethal models involve the development of acute lung infection over 24 – 48 h, which may involve septicaemia. This induces peribronchial and

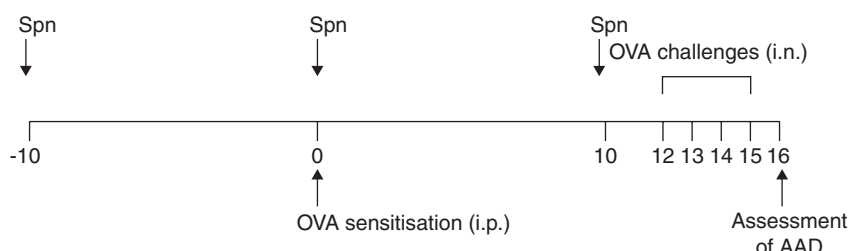


Figure 1. Model of acute allergic airways disease (AAD) and intervention with *Streptococcus pneumoniae* (Spn). Groups are sensitized intra-peritoneally (i.p.) with ovalbumin (OVA) in a T helper type 2 (Th2)-inducing adjuvant. Th2 cells are allowed to develop and AAD is precipitated 12 days after sensitization by four intra-nasal (i.n.) OVA challenges without adjuvant. AAD is assessed 1 day later. Spn exposure can occur before (Phase I), during (Phase II) or after (Phase III) sensitization.

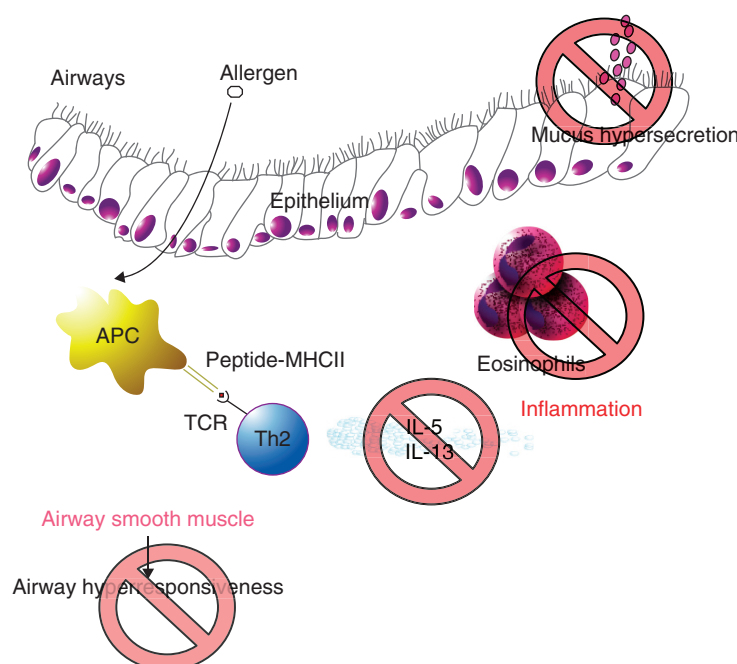


Figure 2. Effects of *Streptococcus pneumoniae* (Spn) on allergic airways disease. Spn suppresses hallmark features of allergic airways disease including airways hyperresponsiveness, T-helper type 2 (Th2) cytokines IL-5 and IL-13, eosinophils and mucus hypersecretion. APC: antigen-presenting cell.

perivascular inflammation and the influx of leukocytes and neutrophils into the lungs, which subsequently resolve the infection.

These models can be combined with allergic sensitization models with infection before (Phase I), during (Phase II) or after (Phase III) sensitization (Figure 1). Thus the development of these non-lethal Spn infection models has allowed the investigation of the effects of infection on the development and progression of allergic airways disease.

12. Spn immunoregulatory therapy: proof-of-concept

The potential for the use of Spn as an immunotherapeutic agent for asthma has been investigated in proof-of-concept

trials [67]. These studies used killed Spn delivered intratracheally to mice at different times relative to sensitization in acute AAD. In order to investigate both prevention and treatment Spn was administered before or during sensitization or before challenge of sensitized mice. Administration at these different time points mirrors the three phases of allergic asthma described above.

Treatment before, during or after sensitization had generally the same effects and suppressed ovalbumin-induced Th2 cytokine release from lung-draining lymph node T cells, eosinophil influx into the airways, lung tissue and blood, mucus secreting cell hyperplasia and AHR (Figure 2). Furthermore these effects were largely reproduced using an extended model of AAD (24 days versus 12 days between sensitization and challenge). This study clearly demonstrates the potential

for the use of Spn as an immunotherapy for AADs and extensive studies are currently underway to further investigate these possibilities.

13. Effect of current vaccines

As a result of the differences in their composition, the currently available 23-valent polysaccharide vaccine (PSv) and 7-valent conjugate vaccine (Cv) elicit different immune responses. PSv generates a T-cell-independent response which is poorly immunogenic, resulting in a low antibody response of largely low-affinity IgM. The lack of conventional T-cell help results in failure to induce antibody affinity maturation, isotype switching or immunological memory [70]. Polysaccharide protein conjugation enables Cv to elicit a T-cell dependent immune response and enhanced antibody production. Therefore, Cv promotes affinity maturation, isotype switching and induction of immunological memory [71]. Unlike PSv, Cv has been shown to induce DC-driven T cell priming [72].

Despite the knowledge of current vaccine efficacy against infection, it is not known whether they may provide an immunoregulatory therapy against AAD. To date, no polysaccharide based vaccine, such as PSv, has been shown to suppress AAD, suggesting that low antibody production is incapable of suppression of the Th2 response. Due to the complex pathogenesis of asthma, we propose that the strong immune response elicited by Cv may encompass the modulatory effects previously shown by killed Spn.

Apart from capsular polysaccharide, additional bacterial components may be involved in modulation of the immune response leading to the suppression of AAD. Identification of the components involved in suppression of AAD by Spn is required to create a specific and targeted immunoregulatory therapy for suppression of AAD. Furthermore, the use of different component combinations will allow us to understand the important underlying modulatory events. The potential for Spn components to work in an independent or synergistic manner in suppression of AAD is unknown.

14. Expert opinion

Asthma is a common disease of increasing global significance. There is a need to understand the genetic and environmental influences that modulate the expression of asthma. Environmental bacteria such as Spn may be able to modulate the expression of asthma, and if so, this effect could be therapeutically useful, using Spn vaccines as immunoregulatory therapy. Future work is needed to identify the efficacy of this approach in experimental systems and to identify the key components of Spn that are responsible for this effect, as well as discovering the mechanism of the effect. Epidemiological studies using existing databases could take advantage of current pneumococcal vaccine strategies to collate human data of the effect of pneumococcal vaccines on asthma prevalence. Once these studies have been completed, a randomized controlled trial of pneumococcal immunoregulatory therapy for asthma is needed to determine the clinical benefits of this approach. Exploiting the beneficial immunoregulatory effects of environmental bacteria represents an exciting approach to address a disease of immense and increasing importance to global human health.

15. Conclusion

Studies indicate that despite the availability of effective pharmacotherapy, asthma remains a common disease with an increasing burden of illness. There is a need to look for other treatment approaches. Reduced exposure to bacteria or their products is associated with increased asthma, and exposure to bacteria, either by vaccination or infection may be protective against asthma. Spn is a potentially useful immunoregulatory therapy for asthma. There is a clear path for future research to evaluate this possibility using model systems, epidemiological studies and clinical trials of available and novel pneumococcal vaccine products.

Declaration of interest

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Bibliography

Papers of special note have been highlighted as interest (*) or of considerable interest (**) to readers.

1. Patel S, Jarvelin M-R, Little M. Systematic review of worldwide variations of the prevalence of wheezing symptoms in children. *Environmental Health* 2008;7:57. Published online 10 November 2008, doi:10.1186/1476-069X-7-57
2. Peat JK, Van Den Berg RH, Green WF, et al. Changing prevalence of asthma in Australian children. *BMJ* 1994;308:1591-6
3. Toelle BG, Ng K, Belousova E, et al. Prevalence of asthma and allergy in schoolchildren in Belmont, Australia: three cross sectional surveys over 20 years. *BMJ* 2004;328:386-7
4. Gibson PG, Henry RL, Shah S, et al. Migration to a western country increases asthma symptoms but not eosinophilic airway inflammation. *Pediatr Pulmonol* 2003;36:209-15
5. Jones PD, Gibson PG, Henry RL. The prevalence of asthma appears to be inversely related to the incidence of typhoid and tuberculosis: hypothesis to explain the variation in asthma prevalence around the world. *Med Hypotheses* 2000;55:40-2
6. Wickens K, Pearce N, Crane J, et al. Antibiotic use in early childhood and the development of asthma. *Clin Exp Allergy* 1999;29:766-71
7. Alm B, Erdes L, Mollborg P, et al. Neonatal antibiotic treatment is a risk factor for early wheezing. *Pediatrics* 2008;121:697-702
8. Verhulst SL, Vael C, Beunckens C, et al. A longitudinal analysis on the association between antibiotic use, intestinal microflora, and wheezing during the first year of life. *J Asthma* 2008;45:828-32
9. Wickens K, Ingham T, Epton M, et al. The association of early life exposure to antibiotics and the development of asthma, eczema and atopy in a birth cohort: confounding or causality? *Clin Exp Allergy* 2008;38:1318-24
10. Miyake Y, Arakawa M, Tanaka K, et al. Tuberculin reactivity and allergic disorders in schoolchildren, Okinawa, Japan. *Clin Exp Allergy* 2008;38:486-92
11. Linehan MF, Frank TL, Hazell ML, et al. Is the prevalence of wheeze in children altered by neonatal BCG vaccination? *J Allergy Clin Immunol* 2007;119:1079-85
12. Arkwright PD, David TJ. Intradermal administration of a killed *Mycobacterium vaccae* suspension (SRL 172) is associated with improvement in atopic dermatitis in children with moderate-to-severe disease. *J Allergy Clin Immunol* 2001;107:531-4
13. Alm JS, Lilja G, Pershagen G, et al. Early BCG vaccination and development of atopy. *Lancet* 1997;350:400-3
14. Strannegard IL, Larsson LO, Wennberg G, et al. Prevalence of allergy in children in relation to prior BCG vaccination and infection with atypical mycobacteria. *Allergy* 1998;53:249-54
15. Aaby P, Shaheen SO, Heyes CB, et al. Early BCG vaccination and reduction in atopy in Guinea-Bissau. *Clin Exp Allergy* 2000;30:644-50
16. Shirtcliffe PM, Easthope SE, Cheng S, et al. The effect of delipidated deglycolipidated (DDMV) and heat-killed *Mycobacterium vaccae* in asthma. *Am J Respir Crit Care Med* 2001;163:1410-4
17. Shirtcliffe PM, Easthope SE, Weatherall M, et al. Effect of repeated intradermal injections of heat-inactivated *Mycobacterium bovis* bacillus Calmette-Guerin in adult asthma. *Clin Exp Allergy* 2004;34:207-12
18. Hirose I, Tanaka H, Takahashi G, et al. Immunomodulatory effects of CpG oligodeoxynucleotides on house dust mite-induced airway inflammation in mice. *Int Arch Allergy Immunol* 2008;147:6-16
19. Gauvreau GM, Hessel EM, Boulet L-P, et al. Immunostimulatory sequences regulate interferon-inducible genes but not allergic airway responses. *Am J Respir Crit Care Med* 2006;174:15-20
20. Schwartz DA, Quinn TJ, Thorne PS, et al. CpG motifs in bacterial DNA cause inflammation in the lower respiratory tract. *J Clin Invest* 1997;100:68-73
21. Bisgaard H, Hermansen MN, Buchvald F, et al. Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med* 2007;357:1487-95
22. Talbot TR, Hartert TV, Mitchel E, et al. Asthma as a risk factor for invasive pneumococcal disease. *N Engl J Med* 2005;352:2082-90
23. Flory J, Joffe M, Fishman N, et al. Socioeconomic risk factors for bacteraemic pneumococcal pneumonia in adults. *Epidemiol Infect* 2008;1-10
24. Almirall J, Bolibar I, Serra-Prat M, et al. New evidence of risk factors for community-acquired pneumonia: a population-based study. *Eur Respir J* 2008;31:1274-84
25. Ansaldi F, Turello V, Lai P, et al. Effectiveness of a 23-valent polysaccharide vaccine in preventing pneumonia and non-invasive pneumococcal infection in elderly people: a large-scale retrospective cohort study. *J Int Med Res* 2005;33:490-500
26. Sheikh A, Alves B, Dhami S. Pneumococcal vaccine for asthma. *Cochrane Database Syst Rev* 2002;CD002165, doi: 10.1002/14651858.CD002165
- **Cochrane review of the use of Pneumococcal vaccines for asthma. Shows that vaccines may be protective but suggests this is due to the prevention of Spn-induced exacerbations rather than immune modulation.**
27. Schuller D. Prophylaxis of otitis media in asthmatic children. *Pediatr Infect Dis* 1983;2:280-3
- **This paper shows that Spn vaccination to prevent otitis media is associated with a 56% reduction in acute asthma attacks.**
28. Spink W. Effects of vaccines and bacterial and parasitic infections on eosinophilia in trichinous animals. *Arch Intern Med* 1934;54:805-17
29. Morgan J, Beeson P. Experimental observations on the eosinopenia induced by acute infection. *Br J Exp Pathol* 1971;52:214-22
30. Bass DA. Behaviour of eosinophil leukocytes in acute inflammation. Lack of dependence on adrenal function. *J Clin Invest* 1975;55:1229-36
- **This paper describes eosinophilia during trichinosis being reversed by Spn infection.**
31. Bass D. Behavior of eosinophil leukocytes in acute inflammation. II. Eosinophil dynamics during acute inflammation. *J Clin Invest* 1975;56:870-9
32. Dietert RR, Zelikoff JT. Early-life environment, developmental immunotoxicology, and the risk of pediatric allergic disease including asthma. *Dev Reprod Toxicol* 2008;83:547-60

33. Afshar R, Medoff B, Luster A. Allergic asthma: a tale of many T cells. *Clin Exp Allergy* 2008;38:1847-57
34. Fanta CH. Asthma. *N Engl J Med* 2009;360:1002-14
35. Abdulmir AS, Hafidh RR, Abubakar F, et al. Changing survival, memory cell compartment, and T-helper balance of lymphocytes between severe and mild asthma. *BMC Immunology* 2008;9:73. Published online 16 December 2008, doi:10.1186/1471-2172-9-73
36. Trautmann A, Kruger K, Akdis M, et al. Apoptosis and loss of adhesion of bronchial epithelial cells in asthma. *Int Arch Allergy Immunol* 2005;138:142-50
37. Lee JH, Yu HH, Wang LC, et al. The levels of CD4+CD25+ regulatory T cells in paediatric patients with allergic rhinitis and bronchial asthma. *Clin Exp Immunol* 2007;148:53-63
38. Akdis M, Verhagen J, Taylor A, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004;199:1567-75
- **This paper highlights the interplay and differences in Tregs between healthy and allergic individuals.**
39. Ling E, Smith T, Nguyen X, et al. Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 2004;363:608-15
40. Karagiannidis C, Akdis M, Holopainen P, et al. Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. *J Allergy Clin Immunol* 2004;114:1425-33
41. Shi HZ, Li S, Xie ZF, et al. Regulatory CD4+CD25+ T lymphocytes in peripheral blood from patients with atopic asthma. *Clin Immunol* 2004;113:172-8
42. Bellinghausen I, Klostermann B, Knop J, et al. Human CD4+CD25+ T cells derived from the majority of atopic donors are able to suppress TH1 and TH2 cytokine production. *J Allergy Clin Immunol* 2003;111:862-8
43. Grindebacke H, Wing K, Andersson A, et al. Defective suppression of Th2 cytokines by CD4+CD25+ regulatory T cells in birch allergics during birch pollen season. *Clin Exp Allergy* 2004;34:1364-72
44. Gambineri E, Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. *Curr Opin Rheumatol* 2003;15:430-5
45. Cottrez F, Hurst SD, Coffman RL, et al. T regulatory cells 1 inhibit a Th2-specific response in vivo. *J Immunol* 2000;165:4848-53
- **In this paper Treg clones are demonstrated to inhibit Th2 responses.**
46. Stock P, Akbari O, Dekruyff RH, et al. Respiratory tolerance is inhibited by the administration of corticosteroids. *J Immunol* 2005;175:7380-7
47. Zhang Q, Fen-hong Q, Hua L, et al. Expression of surface markers on peripheral CD4+CD25high T cells in patients with atopic asthma: role of inhaled corticosteroid. *Chinese Medical Journal* 2008;121:205-12
48. Kearley J, Barker JE, Robinson DS, et al. Resolution of airway inflammation and hyperreactivity after in vivo transfer of CD4+CD25+ regulatory T cells is interleukin 10 dependent. *J Exp Med* 2005;202:1539-47
- **The first research, to our knowledge, to show inhibition of airway dysfunction by transfer of Tregs.**
49. Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 2003;111:1255-61
- **This paper describes successful antigen-specific immunotherapy through the induction of Tregs.**
50. Jutel M, Akdis M, Budak F, et al. IL-10 and TGF- β cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003;33:1205-14
51. Hadley JS, Wang JE, Foster SJ, et al. Peptidoglycan of *Staphylococcus aureus* upregulates monocyte expression of CD14, Toll-like receptor 2 (TLR2), and TLR4 in human blood: possible implications for priming of lipopolysaccharide signaling. *Infect Immun* 2005;73:7613-9
52. Schroder NW, Morath S, Alexander C, et al. Lipoteichoic acid (LTA) of *Streptococcus pneumoniae* and *Staphylococcus aureus* activates immune cells via Toll-like receptor (TLR)-2, lipopolysaccharide-binding protein (LBP), and CD14, whereas TLR-4 and MD-2 are not involved. *J Biol Chem* 2003;278:15587-94
53. Dziarski R, Gupta D. *Staphylococcus aureus* peptidoglycan is a toll-like receptor 2 activator: a reevaluation. *Infect Immun* 2005;73:5212-6
54. Kadioglu A, Andrew PW. The innate immune response to pneumococcal lung infection: the untold story. *Trends Immunol* 2004;25:143-9
55. Dagan R, Melamed R, Muallem M, et al. Reduction of nasopharyngeal carriage of pneumococci during the second year of life by a heptavalent conjugate pneumococcal vaccine. *J Infect Dis* 1996;174:1271-8
56. Malley R, Stack AM, Ferretti ML, et al. Anticapsular polysaccharide antibodies and nasopharyngeal colonization with *Streptococcus pneumoniae* in infant rats. *J Infect Dis* 1998;178:878-82
57. Anderson P, Pichichero MEINSEL. Immunization of 2-month-old infants with protein-coupled oligosaccharides derived from the capsule of *Haemophilus influenzae* type b. *J Pediatr* 1985;107:346-51
58. Ahman H, Kayhty H, Lehtonen H, et al. *Streptococcus pneumoniae* capsular polysaccharide-diphtheria toxoid conjugate vaccine is immunogenic in early infancy and able to induce immunologic memory. *Pediatr Infect Dis J* 1998;17:211-6
59. Kolls JK, Linden A. Interleukin-17 family members and inflammation. *Immunity* 2004;21:467-76
60. Kadioglu A, Coward W, Colston MJ, et al. CD4-T-lymphocyte interactions with pneumolysin and pneumococci suggest a crucial protective role in the host response to pneumococcal infection. *Infect Immun* 2004;72:2689-97
- **This research showed that Treg cells are induced by Spn to a greater extent than by other Gram-positive bacteria.**
61. Akbari O, Stock P, Dekruyff RH, et al. Role of regulatory T cells in allergy and asthma. *Curr Opin Immunol* 2003;15:627-33
- **This paper and [68] describe of chronic models of AAD that are representative of human asthma.**
62. Fattouh R, Midence NG, Arias K, et al. Transforming growth factor- β regulates house dust mite-induced allergic airway

- inflammation but not airway remodeling.
Am J Respir Crit Care Med
2008;177:593-603
63. Zuany-Amorim C, Sawicka E, Manlius C, et al. Suppression of airway eosinophilia by killed Mycobacterium vaccae-induced allergen-specific regulatory T-cells.
Nat Med 2002;8:625-9
 64. Siegle JS, Hansbro N, Herbert C, et al. Airway hyperreactivity in exacerbation of chronic asthma is independent of eosinophilic inflammation.
Am J Respir Cell Mol Biol
2006;35:565-70
 65. Ito K, Herbert C, Siegle JS, et al. Steroid-resistant neutrophilic inflammation in a mouse model of an acute exacerbation of asthma. Am J Respir Cell Mol Biol
2008;39:543-50
 66. Horvat JC, Beagley KW, Wade MA, et al. Neonatal chlamydial infection induces mixed T-cell responses that drive allergic airway disease.
Am J Respir Crit Care Med
2007;176:556-64
 - **This research uses a model of acute phases of asthma.**
 67. Preston JA, Essilfie A-T, Horvat JC, et al. Inhibition of allergic airways disease by immunomodulatory therapy with whole killed Streptococcus pneumoniae. Vaccine
2007;25:8154-62
 - **The first publication, to our knowledge, to show that Spn exposure can suppress AAD in mouse models.**
 68. Gingles NA, Alexander JE, Kadioglu A, et al. Role of genetic resistance in invasive pneumococcal infection: identification and study of susceptibility and resistance in inbred mouse strains. Infect Immun
2001;69:426-34
 - **This paper and [61] describe of chronic models of AAD that are representative of human asthma.**
 69. Preston JA, Beagley KW, Gibson PG, et al. Genetic background affects susceptibility in nonfatal pneumococcal bronchopneumonia. Eur Respir J
2004;23:224-31
 - **A mouse model of non-fatal Spn respiratory infection is described.**
 70. Kato H, Takeuchi O, Sato S, et al. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature
2006;441:101
 71. Goldblatt D. Conjugate vaccines. Clin Exp Immunol
2000;119:1-3
 72. Meltzer U, Goldblatt D. Pneumococcal polysaccharides interact with human dendritic cells. Infect Immun
2006;74:1890-5

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