

BACTERIA AND THE ALLERGY EPIDEMIC: THE CULPRITS AND THE CURE?

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SUMMARY

A reduction in the diversity and magnitude of 'microbial burden' in early life is one factor that has been implicated in the dramatic rise in the propensity for allergy sensitisation. There has been growing speculation that fewer bacteria-derived maturation signals during early immune development increase the susceptibility to allergic responses. Although there is currently no definitive proof of this, evidence that bacteria can inhibit allergic immune responses provides a plausible mechanistic basis for reduced microbial burden as a factor in the current 'allergy epidemic'. Furthermore, the immunomodulatory properties of bacterial products have been utilised in allergen immunotherapy and other treatments for allergic disease with some success, and are a continuing avenue of research. This review discusses the immunological effects of bacterial products including their possible role in emerging models of allergy pathogenesis as well as their associated therapeutic potential in both treatment and prevention of disease.

INTRODUCTION: THE ALLERGY EPIDEMIC

The epidemic rise of allergic disease which is most apparent in 'westernised' countries has occurred in parallel with many societal and lifestyle changes, including improvements in public health, reduction in childhood infection, vaccination programmes, dietary changes and changes in family structure and living conditions. It is self-evident that these or other environmental changes must be responsible for the increasing propensity for allergic disease. The urgent search for causal associations is driven by the need to reverse this trend either by modifying the environmental changes responsible or developing safe and effective strategies to overcome their effects. While there is a virtually endless list of environmental changes which could be implicated, the focus has been on factors which could have plausible influences through known immunological effects.

Among the most plausible candidates has been the apparent decline in exposure to infectious agents and other microbial factors. While the original 'hygiene hypothesis' was based on epidemiological associations this has been consolidated more recently by the more detailed understanding of the immunological effects of microbial factors (discussed below). Although these observations have provided powerful support for the hypothesis that early microbial exposure protects against atopic sensitisation, there is still no definitive proof of this. Because of the therapeutic potential of many inactivated microbial products these

factors have been pursued as methods of modifying allergic immune responses at the individual level, regardless of the speculated effects of changing microbial exposure at the population level. It is already clear that many bacterial-derived products could play a useful role in more definitive treatment strategies or disease prevention (also discussed below).

These concepts need to be considered in the wider complex context of allergic diseases, which are heterogeneous and multifactorial conditions. It appears increasingly likely that different factors (environmental and genetic) are operating in different individuals with different predisposing genotypes. It is therefore logical that optimum therapeutic pathways could also differ in a given population. Future advances in technology will hopefully allow us to more accurately predict allergic risk and to tailor prevention strategies.

IMPORTANCE OF EARLY EVENTS

It is now generally accepted that events during early life when systems and responses are developing are likely to have more formative effects.² This also appears to be true of the immune system. There is still a great deal of uncertainty surrounding the early immune events that may lead to atopy, but there is accumulating evidence of early pre-symptomatic differences between atopic and non-atopic individuals at birth.³⁻¹¹ These differences seem to affect a number of different 'read outs' of immune activity at birth, including the magnitude^{4,12,13} and pattern of cellular responses *in vitro*,^{3,8} circulating neonatal levels of cytokines¹⁰ or cytokine-producing cells¹⁴ *in vivo*, and activity of progenitors that give rise to pro-allergic inflammatory cells (eosinophil progenitors).¹¹ It is not clear if these early differences are merely a detectable measure of increased genetic predisposition, or whether they are indicative of early (*in utero*) environment influences that are already promoting the development of the allergic phenotype. It seems increasingly likely that both are true, particularly as (i) disease is increasing and, (ii) these conditions (such as atopic dermatitis and food allergies) may be manifest within months of life. It is therefore intuitive that the processes that promote allergic inflammation are initiated in this very early period.

Although allergen-specific responses have been detected in fetal life,¹⁵ there has been ongoing speculation about whether allergen-responsive fetal T cells have been primed by antigen exposure *in vivo* or if these reflect some other poorly understood process (reviewed by Prescott and Jones¹⁶). Only half of the allergen-responsive T cells in cord blood have recently been shown to have the CD45RO+ 'memory' phenotype and it is not clear why the remaining responses are being observed in 'naïve' cell populations.¹⁷ For many reasons it may be inappropriate to assume that neonatal CD4+CD45RO+ T cells are equivalent to conventional adult T cell 'memory' cells.¹⁶ Mechanisms of potential *in utero* antigen exposure are also unconfirmed. Environmental proteins have been measured in amniotic fluid,¹⁸ and there is also indirect evidence that these can cross the placenta. Although environmental antigens induce MHC class II dependent cord blood lymphoproliferative responses, this is associated with unusually high levels of apoptosis.¹⁹ Furthermore, the

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surviving cell populations appear to have 'suppressive' or 'regulatory' properties in culture. These cells also express markers which are common (but not exclusive) to T regulatory cells, including CD4, CD25, and CTLA4.¹⁹ The significance of this is still unclear, and further studies are needed. There is obvious interest given the growing focus on the role of regulatory T cells in early immune development (discussed below).

Microbial factors and the perinatal period

There are a number of unique features of immune responses during this early period. Pregnancy is associated with complex interactions at the materno-fetal interface, which reduce cell-mediated tissue rejection and Type 1 (interferon-gamma (IFN- γ) immune responses. An increase in Type 2 immune activity is among a number of mechanisms evolved to protect the fetus in this context.²⁰ At birth, the cellular responses of the fetus continue to reflect this 'Type 2' skewed pattern.⁹ Whether due to immaturity or active Type 1 regulation²¹ (or both), the neonatal capacity for IFN- γ responses is significantly impaired compared with those of adults, resulting in an increased vulnerability to infection during this period. A large number of studies have consistently linked a greater relative immaturity in IFN- γ responses during this period and the risk of subsequent allergic disease.^{3,8} Again, it is not clear if this is purely a reflection of the atopic genotype, or if antenatal factors have inhibited Type 1 immune development to the extent that these individuals are much more likely to progress to persistent Type 2 responses to allergens and associated allergic disease.

In this context it is worth noting that intrauterine infection is associated with increased capacity for neonatal Type 1 responses,²² confirming that antenatal exposures have the potential to influence maturation of Type 1 function. Thus, it is possible that 'cleaner environments' could be having an effect even before birth. There is speculation that non-infectious microbial exposures such as maternal faecal flora might have effects during this period, but this is unconfirmed. One recent report did observe that giving microbial (probiotic) supplements in pregnancy was associated with reduced atopic dermatitis in infancy and early childhood,^{23,24} but the supplementation was also given in the postnatal period and cannot be attributed to an antenatal effect.

Although the expression of bacterial recognition receptors (such as CD14) are reduced in the neonate compared with the adult,²⁵ soluble CD14 has been detected in amniotic fluid²⁶ at lower levels in infants that go on to develop early manifestations of allergic disease (atopic dermatitis).²⁶ This suggests that the highly conserved pathways through which the immune system recognises bacteria might (below) also be implicated in the development of allergic responses. Genetic studies also support a role for the CD14 pathway in the development of allergic disease. Functional genetic polymorphisms in the region of the CD14 gene have been associated with total serum IgE levels,²⁷ and the same group noted that soluble CD14 levels were positively correlated with Type 1 (IFN- γ) responses but inversely correlated with Type 2 (interleukin (IL)-4) responses.²⁷ Although still speculative, it is possible that functional polymorphisms in this pathway confer altered susceptibility to Type 2 allergy responses secondary to altered sensitivity to Type 1-inducing lipopolysaccharide (LPS).²⁸

Microbial factors and postnatal immune regulation

The effects of bacteria on immune development are likely to be greatest in the postnatal period when the infant has more direct contact with environment. The

first years of life see the gradual maturation of Type 1 responses, although this appears not to consolidate until after 18 months of age²⁹ so that responses during this early period are relatively skewed towards the type 2 pattern which normally characterised allergic disease. However, despite this, the majority of infants do not go on to develop atopy. Although it has not been confirmed, there has been longstanding speculation³⁰ that an important contributing factor in the development of Type 2 immune disease is delayed development of Type 1 function in infancy. Bacterial exposure during this period arguably provides the strongest signal for maturation of Type 1 immune function (which is logically involved in defence against these same organisms). A reduction in the level and variety of early microbial burden is an obvious candidate in the search for culprits in the spiralling levels of allergic disease.

The immunological effects of infection are likely to vary according to the type of organism,³¹ the site of infection³² and the genetic susceptibility of the individual. Additional factors that are likely to contribute to allergic risk in this context include the timing of infection and other factors that alter the prevailing environmental conditions or host response. Although most infectious agents provoke strong Type 1 responses, as an essential part of host defence, some pathogens, such as respiratory syncytial virus (RSV), can also evoke Type 2 responses under some conditions. Differences are also seen in the responses to vaccine antigens compared with the wild type infections they prevent.

At this stage there is no clear evidence that infection in infancy either prevents or contributes to the development of allergic disease. A recent large national cohort study (including 24 341 mother-child pairs³³) found that early infections do not protect from allergic diseases such as atopic dermatitis. However, they observed that other indirect markers of microbial exposure (such as early day care attendance, having three or more siblings, farm residence, and pet keeping) were protective. This highlights the emerging concept that overall 'microbial burden' rather than specific infections may be more relevant in early life.²⁸ In keeping with this, it has been noted that non-pathogenic colonising organisms may also play a central role in immune development.³⁴

One observation that remains difficult to reconcile is the increasing number of reports that allergy in childhood is not associated with simple 'Type 2 skewed' immune responses.^{35,37} In these studies, atopic children clearly have stronger Type 1 IFN- γ responses to allergens as well as the characteristic Type 2 responses. These observations suggest that the atopic response may develop as a result of a more fundamental failure of *immune regulation*, rather than a simple skewing of immune response along a Type 1-2 continuum as previously thought. The role of bacteria in this proposed immune dysregulation has not been clear, but new models of allergy pathogenesis (discussed further below) have gone a long way to explain this by proposing that bacteria enhance the activity of many cells involved in regulatory pathways (including but not limited to CD4+CD25+ T regulatory cells, CD8+ T cells, epithelial cells, dendritic cells (DC) and other antigen-presenting cells (APC) (reviewed in detail by Wills-Karp *et al.*³⁶).

EFFECTS OF MICROBES ON IMMUNE REGULATORY PATHWAYS: EMERGING MODELS OF ALLERGY PATHOGENESIS

Activation by microbial exposure appears to be an important stimulus for the maturation of these regulatory cells (above). In an emerging model of allergy

pathogenesis,^{38,39} it has been proposed that environmental changes (such as reduced microbial burden) have led to impaired development of regulatory pathways such that they allow inappropriate responses (both Type 1 and Type 2) to go unchecked. This has been used to explain the paradoxical rise in both allergy (Type 2 immune disease) and autoimmunity (Type 1 immune disease) during the same period.³⁹ This model challenges previous notions that these diseases arise from simple polarisation of T cell responses and suggests that the environmental changes that are leading to the allergy epidemic are not exerting effects through a simple Type 2 polarising influence. Impaired immune regulation could have clear consequences for local and systemic immune development.

Although early research focused on the role of the polarity of T cell signalling as a regulatory factor in immune responses, it is now recognised that DC and regulatory T cells have a key role. In any situation the immune system must tread a narrow path between adequate, defensive responses and inappropriate (pathological) immune responses (as in allergic disease and autoimmunity). In the simplest terms, a major role of DC is to ensure that an appropriate type and level of immune response is initiated. Typically when these cells are activated during infections, they orchestrate both innate and cognate responses. Cells with regulatory properties (including both T regulatory cells and DC) are also activated by the same pathways but provide additional regulatory signals by direct cell contact (CD4+CD25+ T regulatory cells)^{40,41} or by the production of cytokines (IL-10 and TGF- β)⁴²⁻⁴⁴ which inhibit inappropriate or excessive responses.

Dendritic cells

DC play a critical role in programming T cell responses, following their migration-induced maturation in regional nodes.⁴⁵ Animal studies suggest that resting DC stimulate Type 2 immune development unless they receive obligatory Type 1-trophic signals during antigen processing.⁴⁶ These signals may occur under conditions of infection or other local stress,^{47,48} which evoke protective Type 1 effector T cell responses. Foreseeably, variations in local inflammation (such as with infection) could have a key role in determining DC maturation and the subsequent pattern of local T cell responses. Animal studies confirm that local airway DC networks are less developed in infant animals, and display markedly attenuated responses to inflammatory triggers.^{49,50} Similarly human infants do not typically show DC in the airways in the absence of inflammation.⁵¹ However, mature DC do appear in association with severe respiratory infection even at this age.⁵¹ This suggests that local tissue events in infancy can influence the maturation of DC and modify downstream T cell programming in early life. This has renewed interest in the role of infection in early life in the aetiology of allergic asthma.

T regulatory cells

There are heterogeneous groups of CD4+ and CD8+ T cells with suppressive or regulatory characteristics. Although the events leading to the generation of T regulatory populations are still incompletely understood, there is accumulating evidence that the activity of these cells can be influenced by microbial factors.

'Naturally occurring' CD4+ T regulatory cells are derived centrally in the thymus and constitutively express CD25 (the α chain of the IL-2 receptor) and other suppressive molecules including CTLA4.^{40,41} This subgroup of T regulatory cells generally appears to exert suppressive effects by direct cell contact rather than cytokine production. The Foxp3 gene appears to

be a critical regulator of the development of this subgroup of CD4+CD25+ T regulatory cells.⁵² The activity of these cells in the periphery can be influenced by microbial exposure, which can signal through pathogen recognition receptors known to be on the cell surface (below).

T regulatory cells can also be generated in the periphery from either CD4+ or CD8+ T cells under specific conditions. These conditions are dictated by ambient cytokine production by other cells including DC, the activity of which can be modified by bacterial encounter (as discussed previously). T regulatory Type 1 (Tr1) can be derived from naïve CD4+⁴² or CD8+⁴³ stimulated by IL-10 producing DC, and have suppressive effects via high levels of IL-10 (and moderate amounts of TGF- β). Tr1 can also be derived experimentally from CD4+ cells (*ex vivo*) using dexamethasone and vitamin D₃⁵³ or other methods. Other subgroups of T regulatory cells exert potent immunoregulatory properties through the production of large amounts of TGF- β (reviewed by Horwitz *et al.*⁴⁴). These Type 2 regulatory T cells (Tr2) can be derived from CD8+ or CD4+ (also referred to as Th3) T cells.

Thus, microbial elements have the capacity to influence DC and T cells during initial signalling events and modify the resulting effector T cell and regulatory T cell phenotypes.

Microbial signalling through Toll-like receptors: effects on regulatory pathways

Innate immune cells involved in the 'first line' of defence (neutrophils, NK T cells, DC and other APC) are 'hard-wired' to recognise bacterial elements through pathogen recognition receptors. Collectively, these Toll-like receptors (TLR) recognise a broad range of pathogen associated molecular patterns (PAMPs),⁵⁴ although each TLR signals the presence of different microbial components and TLR expression varies with each cell type. Gram-negative bacteria (lipopolysaccharide (LPS)) are largely recognised by TLR4 (in association with CD14). Gram-positive bacterial products (such as peptidoglycan, lipoproteins and lipoteichoic acid) are recognised by TLR2. The main ligand for bacterial DNA (CpG oligodeoxynucleotides [ODN]) is TLR9. Other TLR recognise bacterial flagellin (TLR5) and viruses (TLR7). A more detailed discussion of these and other ligands can be found elsewhere.⁵⁵

CD4+CD25+ T regulatory cells express both TLR4 and 9 (but also TLR5 and 7).⁵⁶ Subclasses of DC also show differential expression of TLR. Myeloid DC (CD14-, CD11c+, CD1a+), found mainly in peripheral tissues, express all TLR except TLR9. Conversely, plasmacytoid DC (CD123+CD45RA+) which are found in circulation blood do express TLR9 (along with TLR1, TLR6, and TLR7) (reviewed by Mazzoni and Segal⁵⁷).

Once activated via these pathways, DC and other APC show enhanced expression of costimulatory molecules and cytokines (including IL-12) which favour Type 1 immune differentiation. This together with enhanced regulatory function may reduce the risk of Type 2 mediated allergic responses^{58,59} during early life when immune maturation is critical. The importance of 'timing' is supported by animal studies, demonstrating that LPS exposure before allergic responses are established can prevent allergic sensitisation.⁶⁰ These effects may be of greater significance in genetically 'allergy prone' individuals who appear to have weaker Type 1 responses in the perinatal period.³⁰

THERAPEUTIC STRATEGIES USING MICROBIAL PRODUCTS

Although the exact contribution of changing microbial burden in the escalating rates of allergic disease is not clear, the immunological effects of these agents have

provided the basis for therapeutic and preventative strategies, which mimic the natural processes of microbial exposure.

Bacteria as vaccines/adjuvants

Vaccine targets need to evolve over the next century to reflect changing disease profiles. With the exponential rise in a wide range of immune-mediated diseases (including many allergic and autoimmune diseases),³⁹ vaccines to prevent these non-infectious conditions are already being developed. A better understanding of the effects of environmental changes on immune development is clearly also essential to understand the reason for these emerging disease patterns as infectious diseases become less prevalent.

In existing infant vaccines to protect from infectious diseases, bacterial-derived adjuvants have been used extensively to enhance immune responses. While adjuvants are very broadly regarded as Type 1 or Type 2 promoters, responses are often mixed and not exclusively polarised. However, broadly speaking, bacterial adjuvants such as immunostimulatory sequences (ISS), unmethylated cytosine and guanosine CpG motifs have more 'Type 1 polarising' effects in mature individuals. Although these are not used in current vaccines there has been growing interest in the potential role of these adjuvants in promoting perinatal Type 1 responses. This is clearly of interest not only in enhancing early protection from infection, but also as a strategy to inhibit the development of Type 2 allergic responses in high-risk infants. This remains largely theoretical at present, and there is ongoing concern about other unpredictable effects on the developing immune system. Recent studies in animals suggests that 'Type 1 adjuvants' (such as complete Freund's adjuvant [CFA]) have paradoxical effects in neonatal animals⁶¹ with inhibition of murine Th1 responses (IgG2a, IL-2, and IFN- γ responses) and enhanced Type 2 (IL-5) responses. In human neonatal mononuclear cell cultures, we recently demonstrated that CpG motifs not only enhance Type 1 IFN- γ responses to vaccines antigens (and other environmental proteins such as house dust mite antigen), but also result in significantly enhanced type 2 responses to these proteins. This suggests that CpG may not have the same Type 1 polarising effects in neonates, and needs to be investigated further.

In established allergic disease, bacteria have been also used as adjuvants in allergen immunotherapy for many decades. These preparations were originally crude and the mechanisms were then uncertain.⁶² It is now known that these effects are probably mediated by CpG and other specific components that signal via TLRs (above). There are now many studies which demonstrate that CpG ISS-ODN inhibit airways inflammation, eosinophilia, IgE responses, Type 2 cytokine levels (IL-5) and bronchial hyper-reactivity and remodelling.⁶³⁻⁶⁶ In mice, the administration of ISS-ODN coupled to ragweed allergen (Amb a 1) induced a Type 1 sustained biased (IFN- γ) response to Amb a 1, with concurrent IgE suppression.⁶⁷ However, it appears that this strategy will be more effective in down-regulating developing rather than established immune responses.⁶⁷ Although still experimental, CpG-allergen immunotherapy is already showing promise in humans. Conjugation of CpG to ragweed allergen produced a large bulky molecular structure, which is less likely to bind IgE, but remains immunogenic. One study in humans has already documented that administration of six doses of this construct lead to a significant reduction in symptom scores in ragweed sensitised patients during the ragweed season.⁶⁸

The role of gut microflora

Intestinal microflora are arguably the most abundant source of early immune stimulation, and contribute significantly to 'microbial burden' in early life. The gastrointestinal tract and associated mucosal immune system is dominated by the production of regulatory cytokines (IL-10 and TGF- β) which promote mucosal immune responses and systemic immune tolerance.

There is good evidence in germ-free animal models that bacterial gut colonisation is essential for maturation of immune function and induction of oral tolerance.⁶⁹ Under germ conditions animals have reduced epithelial cell turnover, motility, vascularity, and immaturity of the gut associated lymphoid tissue (GALT), the largest lymphoid collection in the body. In keeping with emerging concepts of immune modulation, these animals are much more prone to unregulated Type 1 responses (autoimmune bowel disease) and Type 2 allergic responses,⁶⁹ suggesting that microflora also have a key role in immune regulation. In humans, it has been proposed that more hygienic environments are also associated with altered gut flora, and modified early immune development. Although there is no conclusive evidence to support this, human studies have shown differences in commensal microbial flora of populations with high atopy prevalence (Sweden) compared with countries with low atopy prevalence (Estonia).^{34,70}

Many groups are now investigating the capacity of probiotic bacteria to modify mucosal immune responses. Probiotics are preparations or components of microbial cells, principally from the genera of *Lactobacilli* or *Bifidobacilli*. The apparent anti-inflammatory properties (reviewed by Kirjavainen *et al.*⁷¹) are still poorly understood and may be due to effects on both specific and nonspecific immunity. There are a number of potential pathways by which probiotics could influence T cell differentiation. This includes activation of APC (via CD14 and TLR pathways) and innate pro-Type 1 immune responses.⁷²⁻⁷⁴

There have been several preliminary studies to address the effects of probiotics on infant atopic dermatitis, one of the earliest manifestations of the atopic phenotype. Two of these reported a clinical improvement in exclusively breastfed infants⁷⁵ or infants with coexistent cow's milk allergy,⁷⁶ who were given a lactobacillus probiotic supplement (*Lactobacillus GG* or *Bifidobacterium lactis*). Although these studies showed promising results in young infants with mild disease (median SCORAD of 16 at inclusion⁷⁵), it has not been clear what effect probiotic supplementation has on older infants with more severe atopic dermatitis. A further crossover study showed a reduction in reported symptoms compared with placebo, although there was a wide age range (1-13 years) and the intervention was not associated with a significant improvement in objectively assessed extent and severity⁷⁷ as assessed using the SCORAD index.⁷⁸

We recently addressed this in a double-blind placebo-control study of 56 children aged 6-18 months with moderate or severe atopic dermatitis (mean SCORAD of 41) using *Lactobacillus fermentum* PCC™ [VRI Biomedical] for 8 weeks (Weston *et al. awaiting publication*). At the end of the study, 2 months after ceasing the supplementation, children receiving probiotics were significantly more likely (92%) to show an improvement in the extent and severity of their lesions (SCORAD) after receiving *Lactobacillus fermentum* PCC™ [VRI Biomedical]. Furthermore, the supplemented group had a significantly lower mean SCORAD index compared with placebo (p=0.01). Further studies are needed to investigate the effects on faecal flora, under-

lying immune responses and the potential long-term benefits on atopic dermatitis and the subsequent development of associated more persistent forms of allergic disease (such as asthma and allergic rhinitis) and aeroallergen sensitisation.

Again, it is possible that any benefits of probiotics will be greatest if given earlier when immune responses are still developing, particularly as interventions (with probiotics) in older individuals with established disease have failed to show any improvement in asthma⁷⁹ or allergic rhinitis.⁸⁰ There has only been one published study to address this issue of primary prevention. The investigators gave maternal probiotics in pregnancy and during the first 6 months of life and demonstrated reduced incidence of eczema at 1 year.²³ Although this effect was still evident at 4 years, there was no reduction in respiratory allergy, IgE levels or allergic sensitisation.²⁴ Effects on underlying immune response were not reported. There are currently insufficient data to recommend this practice but, given the background of evidence, further studies are clearly needed and there are many groups trying to address this.

Other bacterial products used to modify immune responses

As discussed above, the adjuvant properties of bacteria have long been recognised.⁶² Early approaches (as early as 1955) included subcutaneous injection of bacteria (including various mixtures of *Staphylococcus*, *Pneumococcus*, *Streptococcus*, *Neisseria* and *Haemophilus* species) with variable clinical effects on 'infectious asthma' (recently reviewed by Matricardi *et al.*⁸¹). More recently, purified bacterial products have been trialed in combination with allergen immunotherapy extracts. Drachenberg and colleagues⁸² recently used a tyrosine-adsorbed glutaraldehyde modified grass pollen allergen extract containing a 3-deacylated monophosphoryl-lipid A adjuvant. They demonstrated that this was efficacious (compared with a placebo containing tyrosine alone) after only four injections. This extract is now commercially available.⁸² A reduction in the number of injections required during immunotherapy using this kind of strategy would be clearly beneficial and needs to be explored further.

As potent Type 1 immunostimulants, mycobacteria antigens have also been considered as agents for both prevention and treatment of allergic disease (reviewed by Beasley *et al.*⁸³). Mycobacteria have also been associated with improved pulmonary function in sensitised animals,⁸⁴ and reduced airways inflammation,⁸⁵ eosinophilia⁸⁶ and expression of adhesion molecules.⁸⁷ Although the mechanisms are not clear, there is some evidence in animals that mycobacteria give rise to allergen-specific CD4+CD45B-low regulatory cells which mediate a reduction in airways inflammation through the production of TGF- β and IL-10.⁸⁸ In humans, BCG vaccination has also been associated with reduced total and specific IgE in allergic individuals,⁸⁹⁻⁹¹ and improved lung function in asthmatics.⁹² Intradermal administration of mycobacteria has previously been used therapeutically in children with existing atopic dermatitis with some success.⁹³ In newborns, BCG can influence immune responses to other antigens (vaccine antigens) promoting both Type 1 and Type 2 responses.⁹⁰ Although BCG administration in the neonatal period does not appear to reduce the risk of developing allergic disease,^{94,95} as with many other agents, the role of mycobacterial antigens for disease prevention still needs to be investigated further.

CONCLUSION

Although the capacity for microbial antigens to promote immunological regulatory pathways and Type 1 immune responses is well recognised *in vitro* and in controlled animal experiments, the exact contribution to the escalating rates of allergic disease is not clear. While the debate about the 'hygiene hypothesis' continues, the therapeutic potential of various microbial agents is an ongoing avenue of investigation for both treatment and prevention of allergic diseases. There is also an ongoing need to investigate other complex environmental changes and variable (still poorly defined) genetic predisposition, particularly in early life when the effects are likely to be more profound.

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