Infections in early life and susceptibility to allergic diseases: relevance of hygiene hypothesis

Sharad V. Gangal* and Rohini Chowgule

Institute of Environmental Medicine, Kasturba Hospital, Sane Guruji Marg, Mumbai 400 011, India

Allergic manifestations such as rhinitis, asthma and eczema are increasing all over the world and more steeply in western industrialized countries during the last few decades. The underlying causes are poorly understood. Genetic factors are unlikely to explain the increased prevalence of allergic diseases as genetic shift in the population cannot occur in such a short duration. Changes in lifestyle, living conditions, food habits and environmental factors are implicated in the increased prevalence of allergic diseases. One of the explanations deduced from hygiene hypothesis is that early-life infections may protect against allergic sensitization. Changes in lifestyle have led to decreased exposure of children to certain infections, which is responsible for the increase in prevalence of allergy and asthma. There is growing evidence that under certain situations exposure to microbial products may instead protect against inflammation. This was further supported by cross-sectional studies involving farming and non-farming households in which concentration of endotoxins exposure showed inverse association with allergic manifestations. This article examines the relevance of hygiene hypothesis on the basis of current knowledge of molecular mechanism of allergy and reviews the recent literature on the role of infections in early-life, in maintaining immune homeostasis.

Keywords: Allergy, dendritic cells, early-life infections, hygiene hypothesis.

DURING the last few decades epidemiological data have shown that the incidence of allergic diseases has increased, associated with obvious decrease in infectious diseases, in industrialized countries, while remaining more or less stable in developing countries[1]. In the United States, Canada, UK, Ireland, New Zealand and Australia, the incidence of allergic airway diseases among 13–14-yr-old children is currently the highest in the world[2], and ranges from 22–32%. There are limited data on the epidemiology of allergy or asthma from the developing world, including India. Small surveys conducted in pockets of different states and presented in different conferences, indicated asthma prevalence from 1.5 to 15%. The earliest study[3] in India conducted 35 years ago reported asthma prevalence to be 2.78 in an urban population aged between 30 and 49 years. In a study conducted as a part of the European Community Respiratory Survey, prevalence of asthma in adults aged 20 to 44 years in Mumbai was reported to be 3.5% using clinician diagnosis and 17% by a physician diagnosis and broncho-provocation test[4]. A recent study[5] in school children from Delhi reported asthma prevalence of 10–13%. Other reports indicate a geographical difference in the prevalence of asthma as well as differences in urban and rural population.

In 1989, Strachen formulated hygiene hypothesis based on epidemiological observations and suggested that reduced exposure to microorganisms due to lifestyle changes, in developed countries is associated with increased prevalence of allergic disorders[6–9] and proposed that infections within households in early childhood may protect against allergic sensitization. These findings were supported by other studies as well[10]. The range of microbes implicated includes pathogens, non-pathogens and other microbial products. Even in the developing countries like India, the affluent urban population following Western lifestyle appears to be more prone to allergic risk, resulting in increased incidence of allergic manifestations like asthma in children[5]. It therefore appears that low socio-economic levels, high temperatures, poor housing conditions and lack of enough hygiene still prevalent in East Asian developing countries may predispose inhabitants to infections and helminthic infestations which protect against allergic sensitization. This is probably the reason why developing countries like India do not show similar rise in allergic diseases as seen in the developed industrialized countries. Increase in air pollution, as a result of changed lifestyle may have a role in asthma, which can worsen the clinical status, but correlation between pollution and incidence of allergy or asthma has not been established.

Genetic risk is no doubt an important factor governing susceptibility to atopic diseases, but the short time during which a steep increase in the prevalence of atopic diseases has been registered cannot be explained by the genetic shift in the population. The degree to which genetic and environmental factors influence the susceptibility to allergic diseases is still not well understood.

*For correspondence. (e-mail: svgangal@hotmail.com)

Environmental and lifestyle factors which have undergone rapid changes have therefore been recognized as the cause of the steep rise in allergic diseases. The hygiene hypothesis received considerable attention from the Indian press, reporting that dirty living can actually be healthy. Many people believe that early infections educate our immune system and protect us from allergic and autoimmune diseases, whereas improved general hygiene and control of infections in industrialized countries has led to increased incidence of asthma. Allergy is an immunological disorder. During the last two decades our understanding of the immunological mechanism of allergy has increased considerably. It will be interesting to find if and how infections play a role in immune homeostasis and regulation of allergic inflammatory disorders on the basis of our current knowledge of the mechanism of allergy and asthma.

Allergy manifests in the exposed parts of our body such as upper airways, lungs, skin and gut. It is fairly established that allergies are caused by the interaction of genetic, environmental and lifestyle factors and include diseases like hay fever, rhinitis, asthma, eczema, conjunctivitis and other reactions at the mucosal surfaces, including anaphylaxis. Allergies are caused by aberrations in the humoral immune response. The allergic individual has the propensity to produce excessive quantities of specific IgE antibodies against innocuous environmental substances called allergens, such as pollen spores, dust, danders, insect venom, food, etc. These IgE antibodies bind to mast cells and basophils through high-affinity receptors. This genetic predisposition to produce large quantities of specific IgE antibodies by some individuals is called atopy. The clinical manifestations are initiated by cross-linking of IgE antibodies bound to specific receptors on mast cells or basophils by the re-exposure of specific allergen, leading to degranulation of cells and release of inflammatory mediators and pro-inflammatory cytokines. Antigen-specific immune responses are dependent on B and T cells characterized by their antigen-specific receptors. Unlike B cells, T cells do not directly interact with antigens. They get activated only when the antigen is presented to them by appropriate antigen presenting cells (APCs) along with specific major histocompatibility complex (MHC)–antigen complex.

Th1/Th2 balance and allergy

We now know that T cells orchestrate the immune response. Mosmann and Coffman[11] identified two classes of CD4 T helper cells in mice, Th1 and Th2 cells, on the basis of their cytokine (IL) release pattern. Th1 cells release IL2, interferon gamma (IFNg) and are assumed to play a role in inflammatory delayed-type hypersensitivity, while Th2 cells release IL4, IL5, IL6, IL9, IL13, etc. mediating humoral responses. Both Th1 and Th2 cells originate from the naive T cells which differentiate on initial antigen contact, either to Th1 or Th2. The polarization of T cells involves a cross-regulation. There is a reciprocal inhibition of Th1 and Th2 cells. IFNg released by Th1 cells inhibits Th2 cells, while IL4 inhibits Th1 cell proliferation. In a normal person the Th1 and Th2 immune responses are well balanced. Allergy was thought to be the result of tilting of the Th2/Th1 balance of immune response towards Th2 type, while shifting of the Th2/Th1 balance towards Th1 type, may result in autoimmune diseases. It is now established that allergic individuals have more Th2 cells and high levels of IL4, IL13, and IL5, IL6 levels in their blood. IL4 and IL13 induce specific B cells to undergo isotype switch and produce IgE, which is the key molecule in allergy. Efforts were therefore focused on artificially changing the Th2 response to a balanced Th1 response to alleviate the allergic disorders[12]. How the naïve CD4 T cell differentiates into effector Th1 or Th2 cells is not clearly understood. It is possible that variation in the binding capacity of the T cell receptor–antigen–MHC complex could be one of the signalling mechanisms. Presence of sufficient amounts of certain cytokines such as IL12/IFN gamma produced by the innate cells or through infection, various transcription factors (TF) such as T-bet for Th1 and GATA-3 for Th2 type differentiation, may be the key intracellular factors in signalling.

Role of early-life exposure to infection on CD4 T cell differentiation

Further studies on maternal responses in allergic children have thrown some light on the signals required for differentiation of CD4 T cells into Th1 or Th2 type. Maternal inheritance of allergy and asthma is reported in many studies[13,14], probably due to maternal foetal immune interactions. Several reports have suggested that the Th2 polarized immunological memory to inhaled allergens in atopics is established during infancy or early childhood[15]. The first encounter of T cells with the antigens, which pass through the placenta, occurs in the uterus. The early allergen-specific T cell response is directed by Th2-type cytokines produced by the tropoblast and other cells of the placenta and is weakly Th2 type by default for successful pregnancy[16,17]. These responses are similar to those seen in allergic diseases. However, majority of the infants do not develop atopic diseases. After birth, the weakly primed foetal Th2 cells are exposed to a high concentration of environmental antigens, especially food antigens, leading to the deletion of food antigen specificities during infancy[18]. While postnatal exposure to small quantities of inhalant antigens could result in the re-modulation of these Th2 responses to Th1-like cytokine pattern or locking the Th2-type response boosting the Th2-type immunity as in atopics. Holt *et al.*[19] and several workers have

reported that the postnatal transition of Th1 cell function from the Th2 skewed state, is significantly slower in children with genetic predisposition. The neonatal immune system requires a certain microbial exposure such as childhood infections, which provides natural signal for postnatal maturation of the immune response towards a Th1 type[16,18,20]. If environmental microbial exposure is insufficient, the T helper cell response remains skewed towards Th2 type, thus favouring atopy. These observations support the hygiene hypothesis, i.e. the lack of microbial exposure in infancy locks the immune response to Th2 type leading to allergic diseases. Thus, Th1/Th2 paradigm was linked to hygiene hypothesis.

However, the earlier hypothesis of Th1/Th2 imbalance leading to skewed Th2 responses as the cause of allergy was soon challenged on the basis of the observations that both Th2 (allergic) and Th1 (autoimmune) type of diseases are increasing simultaneously and can be seen in the same patient. It was also revealed that the parasites showing Th2 response paradoxically provide protection against allergy[16]. This means that both Th1 and Th2 responses can increase simultaneously and skewed Th2 response alone may seldom arise in allergic condition. Therefore, some default in the underlying regulatory mechanism seems to allow the unregulated responses of both Th1 and Th2 types. In an immerging model of allergy pathogenesis, it has been proposed that environmental changes have led to impaired development of regulatory pathways, such that they allow inappropriate responses go unchecked[21]. There is a growing appreciation that microbial exposures have a significant influence on immune development and allergic risk.

In the last few years, a variety of T cell subsets have been described which can inhibit effector T cell responses. These cells are called T regulatory (Treg) cells. Majority of Treg cells expressing surface antigen CD25, are programmed in the thymus and are known as natural Treg (nTreg). Other Treg cells derived *de novo* from a naïve CD4 precursor after encountering exogenous antigens in the presence of TGF-B are called adaptive (aTreg) cells. FOXP3, a member of the forked winged helix family of transcription factors, has been reported to be an exclusive marker of nTreg. Both the types of Treg cells, i.e. natural and adaptive, exert their regulatory activity either by cell-to-cell contact (nTreg cells) or by the release of suppressive cytokines such as transforming growth factor (TGF-B) or IL10. Production of IL10 is increased in a number of infectious diseases; probably nature's way to suppress immunopathologic complications. Moreover, $CD4^{+}$–$CD25^{+}$FOXP3 Treg cells were induced by TGF-B in the absence of IL6. TGF-B has been shown to be essential for Treg cell induction, which is expressed in many tissues. There are a lot of data to suggest that infectious agents stimulate the production of regulatory cells which can inhibit both Th1 and Th2 responses[22,23]. These regulatory T cells are involved in the prevention of inappropriate responses leading to allergic diseases. Treg cells predominantly represent the dominant subset specific for common environmental allergens in sensitized healthy individual, while there is a high level of allergen-specific Th2 cells in patients with allergy. The balance between the effector Th1, Th2 cells and Treg cells is governed by antigen-presenting cells such as dendritic cells and environmental conditions. A recent report[24] suggests that IL4 blocks the generation of TGFB-induced FOXP3 Treg cells, and instead induces a population of T cells that produce IL9 and IL10, with no regulatory properties. Runt-related TF1 promotes Treg cells and inhibits differentiation of Th2 cells. An imbalance between Th2 and Treg cells results in atopic diseases. Recently, it has been suggested that lower microbial burden does not act by inducing lower production of Th1-polarizing cytokines but by decreasing the activity of Treg cells, thus tilting the Th2/Treg cell response towards Th2 type[25].

The sensitization and progression towards asthma are fundamentally influenced by innate immune cells; epithelial cells and the induction of adaptive immunity. The APCs, namely dendritic cells (DCs) have an important role in T cell priming and differentiation. DCs that capture the antigens in the periphery are functionally immature and unable to stimulate the T cells[26]. Pathogen associated molecular patterns (PAMPs) consisting of conserved microbial structures constitute a major DC maturation factor. PAMPs are not expressed by mammalian cells and are conserved across a wide range of pathogens. DCs express many pattern-recognition receptors, including toll-like receptors. Recognition of PAMPs takes place through these toll-like receptors and represents a link between the innate and adaptive immune system. Microbial stimuli accompanying a particular antigen lead to maturation of DCs and migration to lymph nodes, and can profoundly change the type of Th response being induced depending upon the dose or type of proliferative infection[27]. Signal pathways activated by toll-like receptor (TLR) ligands lead to cytokine gene transcription and expression of co-stimulatory molecules.

At present, 11 mammalian TLRs have been identified which recognize different ligands. TLR2 binds peptidoglycans, a constituent of the bacterial cell wall. TLR4 recognizes most species of lipopolysaccharides (LPS)[28]. Different TLRs interact with different PAMPs and respond to a number of microbes. Signal pathways activated by TLR ligands lead to cytokine gene transcription and expression of co-stimulatory molecules on the DCs.

Under normal conditions, the usual outcome of inhalation of harmless antigen is the induction of tolerance, because partially mature DCs presenting the antigen induce an abortive proliferative response of unfit T cells which cannot survive and are deleted[27]. Others have reported that partially mature DCs that express co-stimulatory molecules ICOSI and secrete the immunoregulatory cytokine IL10, induce the formation of Treg

cells. In recent years, DC populations have been split up into a number of defined subgroups, of which conventional DCs and plasmacytoid DCs (pDCs) are the main subsets. Conventional and pDCs handle the MHC–antigen complex differently after activation. pDCs have been identified in the lungs, which are crucial for maintaining tolerance. In homeostatic conditions, a fine balance exists between the various functions of lung DC subsets[29].

When full DC maturation is induced in the lungs due to very high doses of endotoxins/lipo-polysaccharide (LPS – a component of the bacterial cell wall) or other microbial motiffs at the time of antigen exposure, a stable Th1 immunity develops in the lungs. High doses of LPS along with inhaled allergens favour the formation of protective Th1 cells or even a regulatory T cell response by stimulating the production of IL12 from the DCs. It has been demonstrated that exposure of DCs *in vitro* with LPS or killed mycobacteria abolished the potential of the DCs to induce Th2 response, due to the fact that a high dose of LPS induces predominantly Ig2a production, preventing allergen sensitization. High level of infection is reported to be associated with higher levels of interferon gamma (IFNg) in the bronco-alveolar lavage and increased production of IL12 by the DCs[30]. Following exposure to PAM patterns that act through TLRs DCs can produce cytokines of the IL12 family[31], IL-12p70, IL23, IL27. Distinct combinations of transcription factors (TFs) have been shown to regulate the expression of genes that encode these three cytokines. The relative proportions of these three cytokines produced, may control the type of immune response depending on the nature of the microbial ligand[31]. However, low-dose endotoxins favour Th2 cell immunity by inducing DC maturation[32,33] in the absence of IL12. A number of reports indicate that the dose of the accompanying TLR agonist is important in determining the type of T cell response. The timing of infection relative to the antigen exposure critically determines whether the allergen sensitization is promoted or suppressed. In the absence of Th1-polarizing signals such as IL12, the Th2 response prevails. In short, the signalling pathway to the development of Th1 cells is induced by pathogens that stimulate IFNg and IL12 production by APCs[34]. Exposure of naïve Th cells to IFNg during TCR engagement with the antigen, activates the TF, signal transducer and activator of transcription (STAT)-1, which in turn activates the downstream TF, T-bet, that is considered as the primary regulator of Th1 differention from CD4 Th cells. T-bet is essential for the genetic programme of CD4 Th1 cell differentiation. Deficiency of TF STAT-1 and IFNg receptor results in defect in T-bet expression and Th1 differentiation. Alternatively, IL12/TF STAT-4 can induce IFNg. Whether the IFNg STAT-1 or IL12 STAT-4 circuit will initiate Th1 differentiation may depend on the capacity of a microbial agent to mobilize the production initially by the innate immune system of IL12 from DCs versus IFNg by natural killer cells[35].

Defects in Th1 cell development and IFNg production result in skewing and over production of Th2 cytokines leading to atopic diseases. There is evidence to confirm that immunity to aeroallergens may result from concomitant exposure to low levels of microbial products, which results in DC activation and Th2 priming or could be due to proteolytic activity within the allergen.

Differentiation of naïve $CD4^+$ cells into Th2 cells is initiated by IL4/IL4 receptor/STAT6 axis, which increases the expression of GATA-3. The GATA-3 binds to target sequences of both Th2 and Th1 cytokine genes, promoting the expression of Th2 and suppressing Th1. Continued expression of GATA-3 is required for the effective maintenance of Th2 cell differentiation. How the signalling and transcription network generate effector T cells has been reviewed in detail by Chatila *et al.*[35].

Another effector T cell lineage has been identified called Th17 cells. These are characterized by the production of IL17 and other related cytokines to constitute the IL17 family. Although the exact role of Th17 in atopy/asthma is not clear, it may contribute to the pathogenesis of asthma by inducing neutrophilic inflammation depending on the route of sensitization. Although IL17 is essential during allergen sensitization to establish allergic asthma, it seems to have a dual role, i.e. stimulation of pro-inflammatory cytokines and downregulation of chemokines in fibroblast with inhibitory effect prevailing at low concentrations. IL17 is reported to have inhibitory effect on DC maturation and the local production of TH2 cytokines IL4, IL13and IL5 in the lungs[36]. The development of Th17 cells shares a common requirement with Treg cells for TGF-B. TGF-B alone can induce Treg cell differentiation; however, in the presence of IL6, TGF-B leads to Th17 commitment. In humans, IL17 appears to be dependent on IL1 B and either IL23 or IL6. Th17 cells express the specific TF retinoid-related orphan receptor (ROR) – that is necessary for Th17 differentiation. Whether the immune response will be dominated by pathogenic Th17 cells or protective Treg cells is governed by IL6, whose expression is classically induced by the action of microbial products on the innate immune system. The exact role of IL17 in allergy and asthma is not clear. In experimental models, exogenously administered IL17 reduces pulmonary eosinophil recruitment and bronchial hyper-reactivity[37], suggesting a regulatory role of IL17. Under physiological strong inflammatory chronic conditions, *in vivo* the number of Treg cells present in the vicinity of an ongoing infection may not be sufficient to skew T cell differentiation, as IL4 may also abrogate the function of Treg cells by inducing the formation of T cells producing IL9 and IL10 with no regulatory properties. Treg cells could promote the development of Th17 cells and production of IL17 which downregulates DC-derived Th2 chemo-attractant, providing evidence for a novel feedback mechanism by which Treg cells may control a Th2 response in the effector

phase of allergic asthma[36]. Thus PAMPs and other microbial products seem to have a profound influence on the development and maintenance of T cell homoeostasis. Thus hygiene hypothesis may yet hold true; the proposed mechanisms are more complex than originally proposed.

There are many studies suggesting that bacterial infections or exposure to bacterial products can inhibit the development of allergic disorders[38,39]. An inverse correlation between the prevalence of asthma and reported rates of tuberculosis (TB) has been found by von Mutius *et al.*[40]. In Japan, Shirakawa *et al.*[41] reported that a positive tuberculin test result suggestive of past infection with TB was inversely related to the development of atopy and asthma. However, a few reports[42,43] could not confirm the findings and the question arose whether the effect of BCG or the negative tuberculin response in vaccinated infants is due to high Th2 propensity and failure to develop Th1 response. Later studies from Guinea-Bissau reported that African children who received BCG vaccination early in life were protected from developing atopy[44]. Whether ethnicity of the population and other environmental exposures play a part is not well understood[45]. BCG infection-suppressed allergic sensitization has been supported by animal model studies[46–48]. Bacteria do not have to be alive to prevent allergic responses. Administration of *Mycobacterium vaccae* during allergen sensitization or challenge induced allergen-specific Treg cells and produced both IL10 and TGFb (ref. 49). Moreover, in India BCG vaccination has been shown to be associated with reduced allergic risk by some workers in retrospective studies. A recent study from Manchester, England, demonstrated an association between the prevalence asthma symptoms and neonatal BCG vaccination, relating to a possible 27% reduction in prevalence[50]. Other bacterial species for example killed *Listeria* species were found to be potent adjuvants for the induction of Th1 response in mice. Bacteria and their components induce either immune suppression or in some cases immune deviation.

Beneficial effects of exposure to certain bacterial infections towards susceptibility to asthma are supported by the observation that administration of antibiotics increases the risk of developing asthma in genetically predisposed children[51]. Epidemiological studies have identified a correlation between the use of antibiotics in the first year of life and increased risk of developing asthma and allergic disorders in children who are predisposed to atopy[52,53]. However, it has been reported that children with pre-existing symptoms of asthma may receive more antibiotics because of their disease and that antibiotic use early in life might just uncover the underlying genetic susceptibility. More recent studies suggest that respiratory-tract infections with *Chlamydia pneumonie* and *Mycoplasma pneumoniae* might facilitate allergic airway diseases[54]. However, airway sensitization depends on the severity and timing of the infection, low-dose infection and antigen exposure-induced allergic airway sensitization, whereas high-dose infection and antigen exposure later did not induce allergic airway sensitization.

Other components of bacteria such as unmethylated cytidine-phosphate guanosine sequences (CPN ODN) present in greater number in bacterial DNA are sensed by TLR9 on DCs and potentiate Th1 immunity[55]. Administration of CPN ODN along with allergen has been suggested for immunotherapy to reduce allergic asthma[56].

Stimulation of innate immune response by microbes, consumption of raw farm milk[57] and helminthes has been investigated. Braun-Fahrlander and his team reported that children whose parents were farmers and who lived on the farms were less susceptible to allergies than those from the same rural region, but not raised on farms[58–61]. This was supported by several studies from other European countries, which suggested endotoxin as the preventive factor. Endotoxins seem to have a dual effect depending on their levels in the environment[62,63]. Recent studies have revealed that the protective effect of farm exposure was confined to Th2-dependent IgG1, IgG4 and IgE expression. These responses are allergen- and switch-specific, which suggest that distinct mechanisms regulate individual steps within allergen-induced class switch *in vivo*. Endotoxins are known to signal through CD14 and TLR9. Specific CD14 single nucleotide polymorphism genotypes are linked with more severe forms of asthma. In severe asthma the production of CD14 and CC16 is increased in an attempt to control airway inflammation and for subjects whose genotype prevents this increase, the ability to control airway inflammation is impaired, resulting in more severe asthma[64]. Growing up in farms is associated with exposure to endotoxins, helminths, lactobacilli and saprophytic mycobacteria, and high levels of mould components, which may protect from developing atopic diseases[65].

The effect of helminths infection on the development of atopy is controversial. Several studies have shown that although helminths infection and allergy have similar immunological make-up, helminths infection paradoxically prevents sensitization from environmental inhalants allergen[35]. Helminth infections are associated with highly polyclonal IgE, which is not specific for parasite antigen. The burden of chronic infection may determine whether it will act as a risk factor or protect against allergic diseases. A variety of parasite infections are associated with generalized immuno-suppression not related to parasite immune response and may protect from the development of atopy[66]. Helminths infection enhances the secretion of Th2 type cytokines and also secretes potent immunomodulatory molecules which may induce T cell hyporesponsiveness[67]. Identifying the distinct parasite molecules that have immunomodulatory effects will help us combat allergy.

Helminths infection elicits a regulatory T cell population able to regulate allergen-induced lung pathology[68,69]. Treg cells characterized by expression of IL2 receptor

(CD25) and the TF-FOXP3 are generated upon parasitic infection controlling both the TH1 and Th2 responses, thereby protecting against allergic diseases in the IL10-independent manner[66]. Helminths parasite infections are known to induce high levels of IgG4, another Th2-dependent isotype which can inhibit IgE-mediated degranulation by effector cells. However, a recent report indicates that sensitization to the helminth *Ascaris lumbricoides* was associated with increased airway responsiveness and hospitalization for asthma[70], which might be related to severity of the infection.

Gut microflora and the risk of allergy

In addition to respiratory route, the oral route of exposure to microbes appears to be common. A number of reports demonstrate that food and orofaecal pathogens such as Hepatitis A, *Toxoplasma gondii*[35] and *Helicobactor pylori*[71] reduce the risk of atopy. This protective effect has been revealed from data from the US NHANES III survey, involving a large number of subjects of various age groups. Clinical or subclinical infection by *Salmonella* in infancy has been reported to contribute to atopy protective influence of a traditional farming environment. However, some gastrointestinal bacteria such as *Campylobactor jejuni*, *Yersinia* and *Clostridium difficle* were associated with a higher prevalence of atopy[16]. These findings raise the point whether gastrointestinal differences in bacteria could be responsible for the development of allergy[72]. However, other investigators[73] could not identify any specific intestinal bacteria associated with the development of sensitization to food in atopic eczema. The microflora hypothesis[74] proposes that perturbations in the gastrointestinal microbiota because of antibiotic use and dietary differences disrupt the normal microbiota-mediated mechanisms of immunological tolerance in the mucosa, which has led to an increase in the prevalence of allergic diseases. It is now becoming clear that balanced microbiota plays a positive role in maintaining mucosal immunological tolerance. Altered microbiota can promote the development of allergic respiratory diseases.

According to the microflora hypothesis, microbiota disruption involves a disruption of this anti-inflammatory environment of the GI tract, where inhaled/swallowed micro-particulate antigens are acquired by the DCs. The composition of digestive flora of newborns in whom allergy develops later, differs from those in whom atopy does not develop. Atopy was associated with increased levels of aerobic microbes and decreased levels of anaerobic microbes in faecal samples. This could be because of the decrease in short-chain fatty acid production by probiotic bacteria, as these short chain fatty acids are known to possess anti-inflammatory properties. The mechanism by which microbiota modulates host immunity is not known; microbial oxylipins could be a potential set of imunomodulatory molecules that could control mucosal tolerance[74]. Probiotic supplementation has again attracted attention with reference to the treatment of allergic diseases. Probiotic supplements are now commercially available. Probiotics are live microbial supplements that exert a beneficial effect on health. The majority of human trials has focused on neonatal or infant subjects. Various studies have demonstrated the ability of probiotics in decreasing severity of atopic eczema in infants[75]. Clinical effects of probiotics have been associated with increased IFN gamma responses in very young children. Although in patients supplementation with curds containing lactobacillus decreased pro-inflammatory cytokine levels, but no clinical improvements were seen. This may be because probiotics have the potential to suppress allergic response before allergic disease is established[76]. One type of probiotic may not be effective in all cases.

Other infections caused by some fungi and parasites have a positive role in the development of allergic disorders. Fungal allergens, including *Candida albicans* are associated with asthma and severe eczema. However, it has been shown[77] that indoor exposure to high levels of fungal components such as cell wall 1,3-β-D-glucan is associated with decreased risk of recurrent wheezing among infants born to atopic parents. Clinical trials conducted by Japanese workers[78] using oral dose of β-1,3-glucan have also shown alleviation of cedar pollen-induced allergic symptoms. Concentration of β-1,3-glucan seems to be critical for the effect.

Protozoans are another group of pathogens which produce Th2 responses. However, their role in etiology of allergy is not clear, although *Nippostrongylus* and *Burgia malayi* have been shown to induce airway hyper responsiveness in mice.

Viral infections and allergy

Although bacterial, fungal and helminth infections under certain conditions have a protective role against allergic diseases, the effect of viruses on the susceptibility to allergic diseases is still controversial.

The immune response induced by viral infection is generally characterized by activation of NK cells and CD8 and CD4 T cells secreting IFNg by APCs. Since IFNg has the potential to inhibit Th2 responses, it is possible that viral infections in early childhood could prevent development of asthma in later life[29]. Although the effect of measles vaccination on the development of atopy is controversial[79,80], recent studies by Marie *et al.*[81] have shown that measles infection can induce immune suppression by the action of two viral proteins on mononuclear cells. Other viruses such as Hepatitis A and *Adenovirus* have been shown to protect against the development of atopy[82]. Childhood infection such as chicken

pox and common cold have been reported to protect children from later problems.

In several studies an inverse relation between asthma and overall burden of respiratory infection has been reported[83–86]. The hygiene hypothesis suggests that in large families and daycare attendance in early-life, children get cross infections that protect them from developing asthma in later life, but does not account for increasing evidence that certain infections might also promote the development of atopy and asthma. It was pointed out that early RSV infection affects sensitization as measured by skin prick test, but does not affect clinical manifestations of atopic disease[87]. In contrast, recent studies warn that RSV infection might be a risk factor for the development of asthma in later life[88–90]. Earlier reports indicate that inflammation accompanying acute influenza infection greatly exaggerated allergic and respiratory responses. Dahl *et al.*[91] have shown using a mouse model that influenza infection enhances type 1 responses in the lungs, but this worsened rather than inhibiting airway inflammation, as it could not counterbalance the Th2 response. Other investigators[92] have demonstrated that chemokine levels produced during RSV infection determine host response to later immune stimuli in the lungs, with the potential to augment the asthmatic response. Protective or harmful effects of infection are likely to depend on a complex mixture of exposure time, environmental cofactors and genetics. Viral infections of the upper airways are the most prevalent and trigger transient wheeze in infancy, but are unlikely to be the cause of atopy. However, infections of the lower respiratory tract early in life have been identified as risk factors for subsequent development of asthma[93]. During viral infection, the allergic individual experiences greater change in airway responses than non-allergic control subjects, suggesting that host factor influences the effect of viral infection on the lower airway function. Each virus elicits its own characteristic immune response depending on the interacting host factors, and various genetic factors significantly modulate the immune response to viruses. It has now been clarified[94] that for children with maternal history of asthma, daycare in early life had no protective effect on asthma and wheeze at age 6 years. Other studies have shown that the phenotype of respiratory illness and hence the host response rather than the infecting virus are the best predictors of further pattern of respiratory illness. Circumstantial evidence points towards both a potentially causative role as well as possibly protective effect of certain respiratory viruses in the cause of allergic asthma during early-life[95]. It is possible that in young children the cumulative effect of respiratory infection over a longer time may in some way alter the lung environment and reduce asthma symptoms[96]. Recently, Bloomfield *et al.*[97] have thoroughly reviewed the literature revealing complexity of the effect of infection and other hygiene practices in relation to atopy. Since they could not conclude the relationship between hygiene hypothesis and hygiene practices, they recommend a renaming of the hygiene hypothesis as microbial deprivation hypothesis as suggested by others and emphasized that good hygiene practice should not be discouraged. The role of microbes and their products in immune regulation is supported by the current knowledge of immunological mechanism of allergy, although we still do not know the type of microbes, period of exposure, etc. that may be helpful for intervention.

Conclusion

The original hygiene hypothesis predicts that infection could protect against allergy and asthma, and the earlier reported mechanism of asthma based on Th2 skewing of the immune response supported the concept. Our understanding of the pathogenesis of allergic diseases has expanded to include the contribution of Treg cells and the proinflammatory Th17 cells. In parallel, there are many studies showing how microbes and their products influence the development of T cell homeostasis through the innate immune system to affect the transcriptional programmes of Th cell lineages. Although increased prevalence of asthma in industrialized countries is generally linked to a change in lifestyle leading to decreased incidence of exposure to infections, recent observations suggest that certain respiratory pathogens might actually enhance the development of asthma.

Our understanding of the mechanism that protects against the development of allergic diseases and asthma is still limited. Sensitization to allergens and progression towards asthma are influenced by a fine balance between the functions of innate immune cells and the induction of adaptive immunity as determined by the balance between Th2 cells and Treg cells. Since microbes are found everywhere around us, it has been suggested that exposure to TLR ligands through the exposed parts of our body such as the intestines, lungs and skin provides an important link between microbes, normal immune development and the atopic phenotype. Our increased knowledge of the immune mechanism now indicates an important role for early microbial antigenic exposure to drive the immune system training through sequential events. The timing of the infection and severity relative to allergen exposure critically determine whether allergen sensitization is promoted or depressed. Although the effect of exposure to infections early in life is to stimulate a shift away from a predominantly Th2 response, early infection also plays an important role in down-regulation of both Th1 and Th2 responses through the induction of Treg cells. It is suggested that reduced exposure to infectious agents in early childhood might exert a profound adverse effect on the developing immune system. We still need to know a lot about the protective factors in our environment which can be used for interventions in Th2 responses acceptable to

our current lifestyle without compromising the essential hygiene and reduce the economic burden of the disease.

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