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Review

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Asthma and allergy—disorders of civilization?

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Introduction

There is both increasing public concern over the rising trends in allergic diseases, and continuing confusion over precisely what is covered by this term in clinical practice. This review examines the cellular and mediator mechanisms involved in the most frequently encountered allergic disorders, especially those involving the respiratory mucous membrane. It stresses the importance of the environment and our effect thereon as a key factor in the rising trends in both the developed and the developing world.

'Allergy' is frequently used loosely to describe human intolerance to environmental factors. This broad use has included such diseases as migraine, irritable bowel syndrome, chronic fatigue syndrome (ME) and, at the extreme end of the spectrum, a 'total allergy syndrome' ('Allergy to the 20th Century'). While a strong case can be made for the human body being intolerant to a wide variety of environmental pathogens and toxins, a more restricted use of the word allergy provides greater insight into one of the most exciting areas of modern medicine. Here, I will narrow C. Von Pirquet's original description of allergy (1906): 'The ability of animals and humans to develop altered responses to foreign substances after repeated exposure' to that of P.G. Gell and R.R. Coombes (1964): 'Immune responses which give rise to irritant or harmful reactions'. A key feature of the allergic response is the involvement of the immune system, which can be exquisitely sensitive to, and specific for, factors in the modern environment.

The classical work of Gell and Coombes[1] divides allergic responses or hypersensitivity reactions into four subcategories on the basis of the timing and type (i.e. antibody- or cell-mediated) of immunological response produced. Since most of the classical allergic responses fall into the first category of immediate (or type 1) hypersensitivity, which differentiates them from those with a more gradual onset, this review will focus on type I hypersensitivity. The range of diseases in which immediate-type hypersensitivity is involved is large. Most frequently, this type of allergic response occurs at a mucosal surface, an important interface between the external environment and internal milieux. It gives rise to such disorders as asthma, perennial and seasonal rhinitis (hay fever), allergic sinusitis, conjunctivitis and, in the gastrointestinal tract, food and drug reactions. In the skin, allergic (atopic) eczema and urticaria (hives) represent two extremes of the Type 1 allergic response, the former being a chronic condition with quiet and active periods, the latter being of sudden onset but usually resolving rapidly. In the eye, allergic responses associated with allergic rhinitis or sensitization to cosmetics leads to an acute superficial conjunctivitis, while a more severe and intractable allergic response such as that encountered in vernal and giant papillary conjunctivitis may be sight-threatening through damage to the cornea. One of the most dramatic manifestations of the immediate-type allergic response is anaphylaxis, in which body contact with minute amounts of the offending allergen (e.g. penicillin, bee venom or peanuts) produces cardiovascular collapse, severe bronchoconstriction, urticaria and swelling of the mucous membranes (angioedema) which may require life-saving measures. To place these varied

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disorders into a mechanistic context, it is necessary to have some understanding of the 'key players' involved.

Cells and mediators of the allergic response

Irrespective of the organs in which they manifest, almost all of the above diseases share a single triggering mechanism which underpins adverse responses to specific environmental allergens. This factor is present in the serum and was originally named '*reagin*' by Prausnitz and Küstner, on the basis that Küstner's allergic response to cod fish could be passively transferred to Prausnitz by injection of serum into the skin followed by local allergen to produce a characteristic wheal and flare response.[2] This test for reagin was subsequently named the PK reaction. Forty-five years later, Johanssen in Sweden and the Ishizakas in the US identified *reagin* as a circulating antibody subtype, and renamed it immunoglobulin E (IgE).[3] IgE, like the other antibody classes, is present in the blood of all humans, but in those with allergic diseases, it is usually present in greater amounts and, more importantly, it is directed against specific environmental allergens. The genetic susceptibility to develop IgE antibodies against common allergens encountered in the environment is referred to as atopy, and those clinical disorders are referred to as atopic diseases. One end of the IgE molecule, the Fab region, is designed to bind to specific components (or epitopes) of the offending allergen, while the other end, the F_c region, binds with high affinity to receptors present on tissue mast cells and circulating basophils (Figure 1). $Fc_\varepsilon R1$ consists of four polypeptide chains, $\alpha\beta\gamma_2$. The α chain binds to five aminoacids (330–335) of the C3 domain of the Fc segment to orientate the IgE molecule so that it lies on its side with the allergen binding site facing outwards (Figure 1).[4] Binding of allergen to two or more adjacent α chains results in receptor clustering, and through interactions involving the α and β chains, sets into motion intracellular biochemical events involving phosphorylation and dephosphorylation of a series of proteins that trigger mast cell activation, with the explosive but non-cytotoxic release of a wide range of preformed and newly generated inflammatory mediators (Figure 2). The attachment of IgE to mast cells (sensitization) can be easily demonstrated by pricking a small amount of allergen through the skin of the forearm. A positive response is a characteristic wheal and flare, due to the release of mast cell mediators, the size of the response being proportional to the concentration of allergen-specific IgE present. Allergen-specific IgE may also be detected directly in the serum and quantified using a radio-allergo-absorbent test (RAST) or related immunoassays.

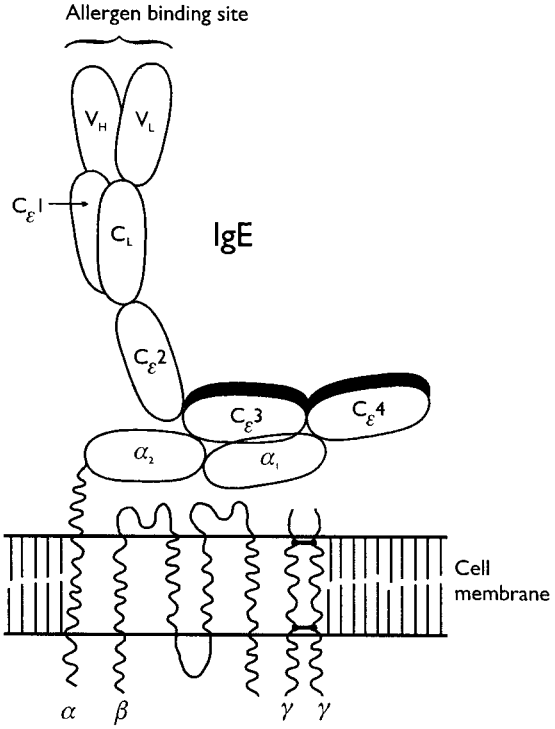

Figure 1. IgE binding to the α chain of the high-affinity receptor.

Within their cytoplasm, mast cells and basophils possess granules which store a number of chemical substances, including histamine, proteolytic enzymes (tryptase, chymase), complex carbohydrate-cleaving enzymes (β-glucuronidase, hexosaminidase, β-galactosidase), the anticoagulant heparin, and cytokines which serve as autocoid and hormone messengers (Figure 2). When activated through perturbation of their IgE receptors (Figure 3), mast cells produce a variety of newly-generated chemical mediators that originate from the mobilization of fatty acids from the cell's nuclear and plasma membranes, followed by enzymic conversion to a group of highly potent inflammatory mediators: the prostanoids (PGD_2, TxA_2) and leukotriene LTC_4, from which two further products derive, LTD_4 and LTE_4.[5] The family of three-peptide leukotrienes (LTC_4, LTD_4 and LTE_4), previously identified in the 1930s by Feldburg, Kellaway and Trethewie as the activity 'slow reacting substance' (of anaphylaxis) (SRS-A),[6] are among the most potent chemical substances known to produce features of the allergic tissue response. LTD_4 and LTE_4 are derived from the extracellular cleavage of the three amino acids, glutathione side-chain of LTC_4 yielding the cysteine-glycine conjugate LTD_4 with further cleavage to the cysteine conjugate LTE_4. Interaction of these different preformed and newly-generated autacoid mediators with specific receptors

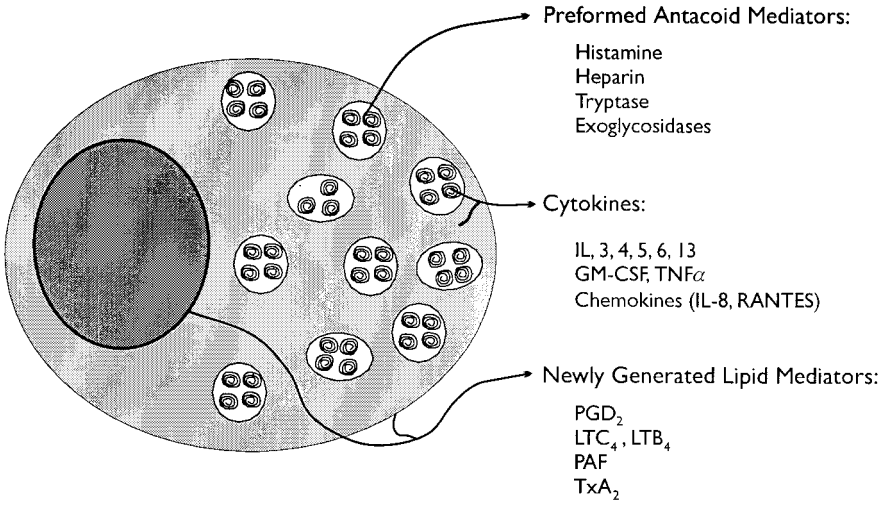

Figure 2. Preformed and newly-generated mediators released upon IgE-dependent activation of mast cells.

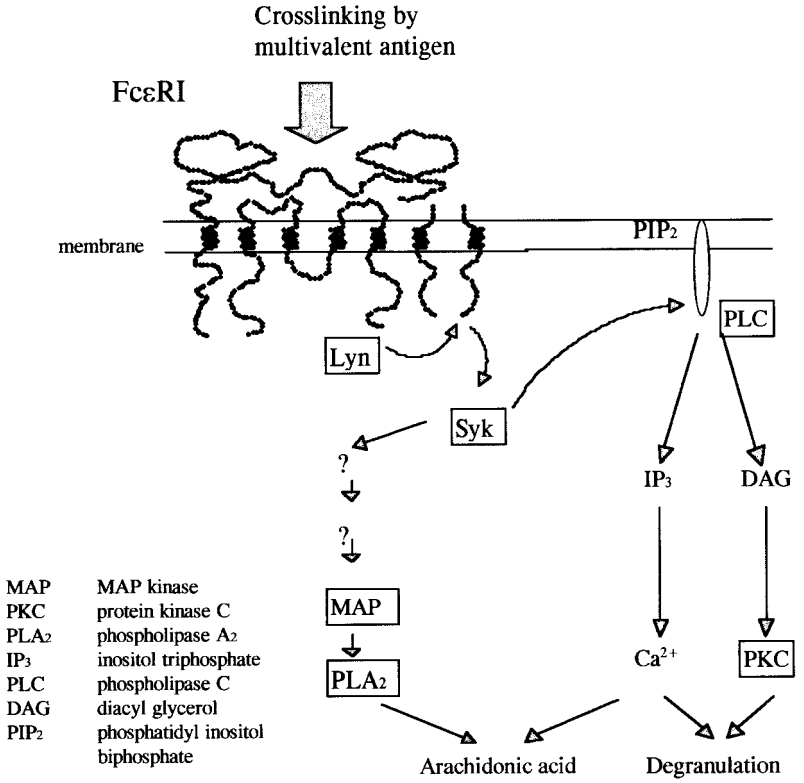

Figure 3. Intracellular events leading to mast-cell mediator secretion upon allergen cross linking of IgE bound to the high-affinity receptor $Fc_{\epsilon}R1$.

on the target tissues produces the acute allergic symptoms, which include contraction of airway smooth muscle, leakage of small blood vessels causing swelling, stimulation of glands to secrete excess mucus, and irritation of nerve endings to create the symptoms of itching and sneezing and cough. When mast-cell and basophil activation occurs in response to *circulating* allergens, then life-threatening anaphylaxis ensues.

In addition to an immediate component initiated by mast cells, many of the allergic disorders have a subacute and chronic component. The cells largely responsible for these are eosinophils, which are selectively recruited into the inflammatory site from the microcirculation of the tissue involved (Figure 4).[7] These bone-marrow-derived cells are selectively removed by the small blood vessels, whose endothelial cells on the luminal side of the lining express molecules that adhere to complementary ligands on the passing leukocyte.[8] Mediators from mast cells,

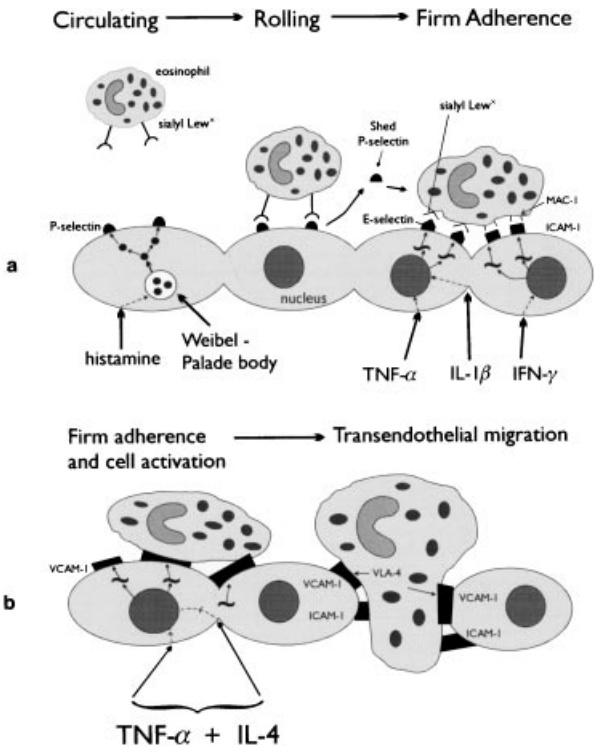

Figure 4. Recruitment of eosinophils from the microvascular circulation involving leukocyte-endothelial adhesion. The selectins (P and E) interact with complex sugar residues on the passing leukocyte to produce 'rolling' along the endothelium. Subsequent cytokine-induced upregulation of the endothelial cell adhesion molecules, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, through interactions with their respective integrins (Mac-1 and VLA-4) on the passing eosinophil produces tight adherence, cell activation and transendothelial migration.

such as histamine and leukotrienes, initiate this adhesion process by increasing the surface expression of P-selectin, which in endothelial cells is stored preformed in Weibel-Palade bodies. Expression of a second set of adhesion molecules: E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), is required for firm adhesion and transendothelial migration (Figure 4). These adhesion molecules are under the regulation of selective cytokines, including TNFα, IL-1β and IL-4, that are released from mast cells and other resident cells in the first few hours of allergen exposure.[9] The leukocyte, while in contact with the endothelial cell, picks up additional signals from cytokines and a range of chemoattractant cytokines (chemokines) which facilitate their migration through the tissue. Further interactions with chemokines, cytokines and matrix proteins slows down the migrating cell and activates it for mediator secretion. In the case of the eosinophil, this includes the release of reactive oxygen, toxic granule proteins, metalloproteases and leukotriene C4.[10]

Orchestration of the allergic inflammatory response

Implicit in the chronic allergic response is a continuous process of IgE generation, mast-cell activation and eosinophil recruitment. These processes are orchestrated by T lymphocytes, cells that play a crucial role in the functioning of the immune response both systemically and at mucosal surfaces.[11] In atopic individuals, T lymphocytes receive an allergen-specific signal from highly specialized antigen-presenting cells (dendritic cells) located at the interface between the external and internal environments.[12] These 'professional' antigen-processing cells take up allergen molecules that land on their surface, by pinocytosis or receptor-mediated internalization, digest them into small peptides, and then present a selected peptide sequence on the surface of the cell, held in the cleft of the proteins that make up the repertoire of the human lymphocyte antigens (HLA or major histocompatibility Class II—MHC Class II) molecules. The presentation of allergen peptides to the T cell usually occurs in local lymphoid tissue, along with essential engagement of co-stimulatory molecules B7 on the dendritic cell and CD28 on the lymphocyte, resulting in the differentiation of the naive T cell to one that generates a range of cytokines which upregulate cells and antibodies involved in the allergic response (Figure 5).[13] The genes for these cytokines are encoded within a small region on the long arm of chromosome 5, a number of them (IL-4, IL-5 and GM-CSF) being co-ordinately regulated. T cells that differentiate along this route, and prefer-

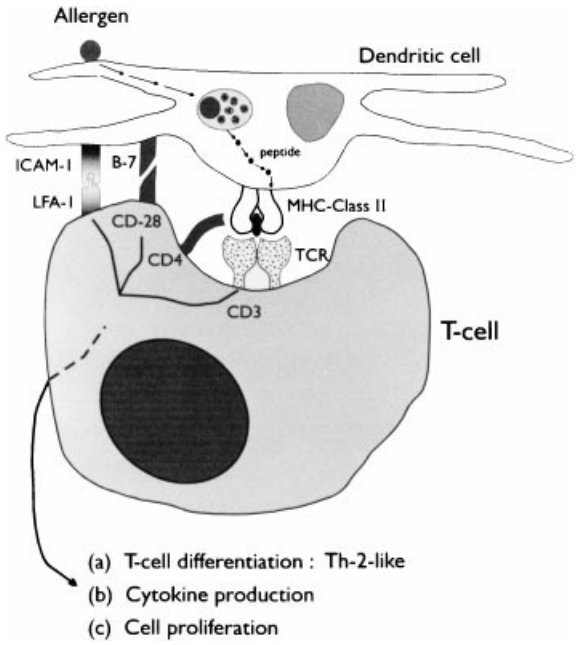

Figure 5. Dendritic cell–T cell interactions leading to allergen-driven cytokine production.

entially release cytokines of the IL-4 gene cluster, are called TH2-like. This differentiates them from a second set of helper T cells (designated TH1-like) which are involved in cell-mediated immunity, a branch of the immune response which protects against viruses, malignancy and intracellular pathogens such as *M. tuberculosis* and *M. lepri,* and which is involved in such chronic inflammatory diseases as rheumatoid arthritis and inflammatory bowel disease. Tissue biopsy studies conducted in patients with allergic diseases in which the cytokine repertoire has been assessed by the presence of cytokine transcription and identification of product release, demonstrate clear over-representation of TH2-like lymphocytes and their cytokines, with their capacity to maintain an ongoing allergic response.

While a number of the TH2-derived cytokines are involved in mast-cell basophil and eosinophil recruitment and maturation, one particular cytokine, IL-4 (and its homologue, IL-13, which shares 30% amino acid homology with IL-4), plays a particularly important role in this arm of the immune response.[15] By interacting with B lymphocytes, these cytokines change the immunoglobulin isotype being secreted from the short-term protective antibody IgM to the allergic antibody IgE. As with dendritic T-cell interactions, effective signalling to B cells requires a cognate interaction with the TH2 cell, involving efficient antigen presentation and engagement of a second set of co-stimulatory molecules, CD40 and its ligand CD40L[15] (Figure 6). Under these conditions and in the presence of IL-4 or IL-13, the B cell splices an ε heavy chain to the immunoglobulin hypervariable region (which is the part of the Fab region that recognizes the allergen) leading to the generation of allergen-specific IgE. If cell–cell contact between the T cell and the B cell is not established, but only IL-4 or IL-13 present, large amounts of IgE which is non-allergen-directed, i.e. non-specific, will be generated. Thus IgE has the important role of linking the recognition of allergen to the signalling of a variety of cells, including mast cells and basophils which express $Fc_{\varepsilon}R1$, leading to the generation and release of a range of highly active chemical mediators. Inhibition of IgE synthesis can be achieved when IgE becomes bound to a second receptor on B cells ($Fc_{\varepsilon}R2$ or CD23) which has a lower affinity for its ligand. The component of the IgE molecule that binds to $Fc_{\varepsilon}R2$ is found in amino acids 367–376, and is therefore separate from the $Fc_{\varepsilon}R1$ high-affinity binding site.[16] Of interest is that the cysteine protease *Der* P_1, the major allergen of the house dust mite, is capable of selectively cleaving cell-bound CD23, thereby depriving the IgE-secreting B lymphocytes of an inhibitory feedback signal leading to augmented *Der-*P_1-specific and non-specific IgE synthesis.[17]

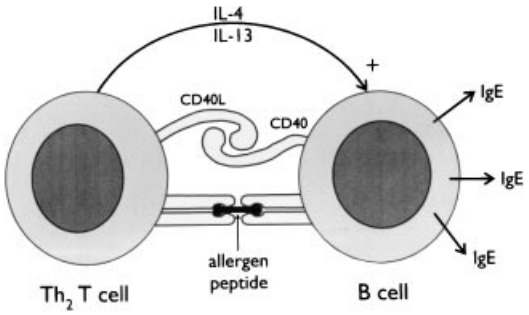

Figure 6. T-cell involvement in the isotype switching of B lymphocytes from IgM to IgE synthesis. CD40L, CD40 ligand.

New therapeutic opportunities from understanding IgE-related mechanisms

A particularly exciting new development with therapeutic potential is the opportunity of removing IgE using monoclonal antibodies of the IgG subclass directed to that part of the IgE molecule that binds to the high-affinity receptors ($Fc_{\varepsilon}R1$) on mast cells and basophils.[18] Thus, when IgE is bound to the mast cell via the 330–335 binding site on the C3 domain of the Fc portion of the molecule, the antibody is unable to find the immunoreactive epitope, because it is engaged in the α chain of the receptor and, unlike allergen or anti-IgE molecules that bind to other parts of the IgE molecule, is unable to crosslink the IgE and, therefore, fails to activate the cells for mediator secretion. However, the antibody is able to bind to IgE both in the circulation and when expressed on the surface of B lymphocytes synthesizing it, to form complexes that can be easily removed. Although these IgG antibodies were originally developed in mice,[18] they have now been 'humanized' so that only a minute proportion of the total monoclonal antibody that is directed to human IgE is of mouse origin, the majority (99%) being human.[19] Phase I clinical studies have already shown that a single injection of humanized IgG anti-IgE can remove IgE from the serum within 30 min, with a duration of up to 30 days.[20] In two separate recent studies,[21,22] administration of a humanized anti-IgE (E25) to allergic asthmatic subjects almost totally blocked the early- (mast-cell-mediated) and late-phase (eosinophil-mediated) bronchoconstrictor responses to inhaled allergen. On the basis that IgE is the principle triggering signal for mast-cell activation, clinical trials are now in progress to see what impact this has on the clinical expression of allergic diseases, including asthma. Naturally-occurring auto-

antibodies against IgE and its high- and low-affinity receptors have also been described.[23] With knowledge of the IgE-$Fc_{\varepsilon}R1$ binding site (amino acids 330–335) it may be possible to develop a peptide vaccine incorporating these amino acids which would artificially induce an autoanti-human IgE that would remove IgE without causing mast-cell activation. Preliminary clinical studies with such a decapeptide in well-defined food-allergic subjects look most promising. Humanized blocking monoclonal antibodies have also been developed against the key allergic cytokines IL-4 and IL-5. Blockade of IL-4 should not only attenuate IgE production, but also reduce the expression of VCAM-1, an essential endothelial ligand for eosinophil recruitment, and inhibit the development of TH2 cells, which are critically dependent upon this cytokine (but, interestingly, not on IL-13). Clinical trials of these antibodies are currently in progress and the results are eagerly awaited.

The immune response to gastrointestinal parasites

The observation that IgE, mast cells, eosinophils and T cells are intimately associated with the pathogenesis of allergic responses, prompts the question as to what the normal function of this system is in the absence of allergic disease. All of these components appear to be involved in anti-parasite responses. Colonization of the gastrointestinal tract with helminth parasites generates a strong TH2 signal, with a massive increase in parasite-specific IgE and in the number of mast cells and eosinophils present within the gastrointestinal mucosa.[24] The presence of appropriate parasite antigens which interact with IgE, initiates chemical mediator release from mast cells and eosinophils, leading to sloughing of the overlying epithelium, contraction of the gastrointestinal smooth muscle, outpouring of plasma proteins, and hypersecretion of mucus, designed to aid expulsion of the parasite. In addition, the eosinophil is well-equipped with parasiticidal molecules, e.g. the eosinophil granule proteins (eosinophil basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin and peroxidase), whose functions are to breach the parasite's integument.[25]

For reasons which are not clearly understood, the parasite killing and elimination response seems also to be used in the development of allergy in tissues distant from the gastrointestinal tract, and in response to environmental allergens. One possible explanation for this transition is that many of the molecules present in sensitizing allergens, such as house dust mites, cat dander and fungal spores, have counterparts in parasites, e.g. the proteolytic enzyme cruzipain in *Leishmania cruzii*.[26] These enzymes appear to be particularly powerful in driving an IgE response, and may in part explain why some proteins are allergenic whereas others are not. It turns out that the major house dust mite, domestic animal and fungal allergens have enzymic functions which may impart particular properties to such molecules, enabling them to breach the protective epithelial barriers by enzymic attack of the cell adhesion molecules that are responsible for maintaining epithelial integrity.[27]

The genetic basis for allergy and asthma

Allergic diseases have long been known to run in families, indicating a strong genetic component. Genetic influences can be divided into two components. The first is the ability of a susceptible individual to recognize a common environmental allergen as foreign, and initiate an allergic immune response. This operates through the human lymphocyte antigens (HLA or MHC Class II) molecules HLA-DR, -DP and -DQ, which provide the mechanism for antigen recognition and presentation to and by T and B lymphocytes.[28] A second set of genetic influences is important in regulating the overall cytokine response. For example, the region on chromosome 5, which contains the IL-4 gene cluster (5q 31–33) in which the 'allergic' cytokines IL-3, -4, -5, -9, -13 and GM-CSF are encoded, is closely linked with the inheritance of an increased IgE response and also to bronchial hyperresponsiveness (BHR), an important physiological marker of asthma. There may be many genes involved in the regulation of TH2 cytokines. A recently identified polymorphism in the IL-4 gene promoter (cytosine to thymidine) increases binding of the nuclear transcription factor NF-AT (nuclear factor of activated T cells) to its palindromic consensus sequence on the DNA promoter, resulting in increased IL-4 production and hence a greater IgE response.[29] However, human genome searches have revealed that the allergic diathesis links with a region on the long arm of chromosome 12 on which there is a gene encoding for interferon-γ (IFN-γ), a powerful suppressor of the TH2 response. There is a reciprocal relationship between TH2 and TH1 responses, IL-10, derived from TH2-like cells, inhibits the TH1 response, whereas IFN-γ, generated by TH1-like cells inhibits the TH2 response (Figure 7). The production of IFN-γ is induced by a further cytokine (designated IL-12) which is released from activated dendritic cells, macrophages and monocytes, and is generated in particularly large amounts during virus infections.[30] Thus, it is possible that, in allergic diseases such as asthma, there is either an increase

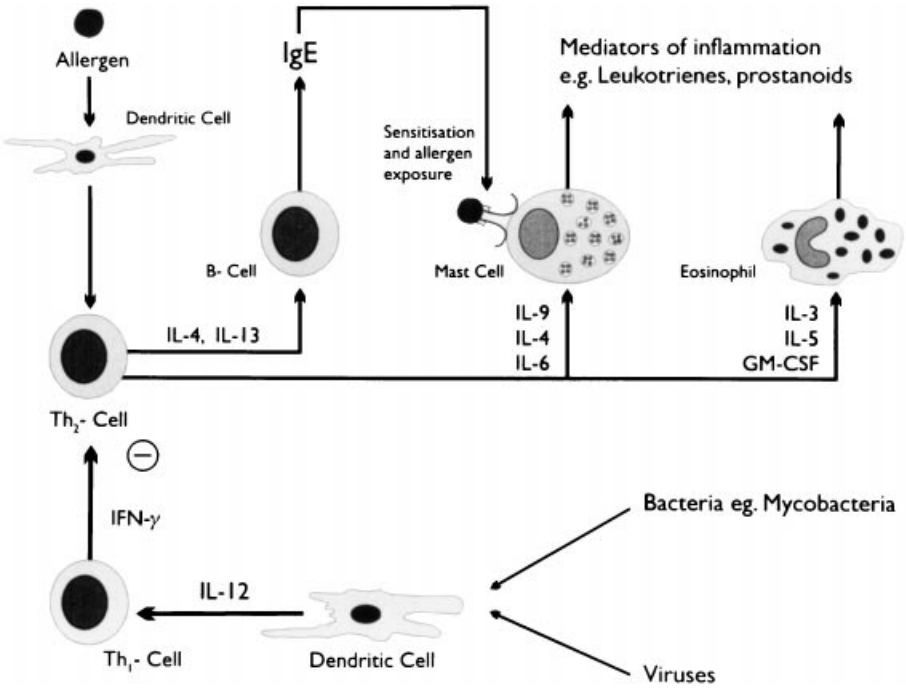

Figure 7. Partition of T-lymphocytes into Th-1 and Th-2 subtypes, dependent upon their repertoire of cytokines.

in the expression of genes which regulate TH2 cytokines or a decrease in expression of genes regulating IFN-γ or IL-12 production, or a combination of both (Figure 7). The next decade is likely to witness an explosion of interest in the genetics of allergy and asthma since identification of susceptibility genes will not only enable individuals at risk of developing disease to be identified, but will also enable preventative environmental or therapeutic strategies to be directed towards them. In discovering novel disease susceptibility genes, there is the hope that targets for new anti-asthma and anti-allergy drugs will also be found.

Environmental factors and allergic disease

While ~40% of the clinical expression of an allergic disorder can be accounted for by genetic factors, for these to be manifest there is an absolute requirement for interactions with environmental factors. The most characteristic feature of the human allergic tissue response is the generation of IgE directed specifically against small amino-acid sequences found on common environmental allergens derived from indoor and outdoor sources. The most important of these include the dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), domestic animals (especially cats, rabbits, horses and rodents) and fungi found in damp housing, e.g. *Cladosporium*, *Alternaria* and *Penicillium*.[31] In non-temperate climates, other allergens may impose themselves on the allergic response. For example, in the poorer districts of cities in North America and in tropical climates, allergens derived from cockroaches seem to be particularly important in initiating and maintaining asthma.[32] On the other hand, where the atmosphere is too dry for the survival of dust mites (e.g. Arizona, Saudi Arabia), allergens derived from fungi such as *Alternaria* and domestic pets become more important in driving allergic responses linked to asthma. In the cooler northern climates, outdoor allergens, such as birch pollen and in the warmer Mediterranean climates, olive and *Parietaria* pollen become important contributors to allergic diseases.[33] However, these outdoor sources of allergen, including those derived from grasses and a wide variety of wind-pollinated trees, appear to be more closely linked to hay fever and allergic conjunctivitis, whereas asthma and eczema are diseases associated with exposure to indoor allergens such as dust mites, animal danders and fungi, although asthma can also be caused by exposure to sensitizing chemicals such as isocyanates, acid anhydrides and platinum salts in the workplace.

Sensitization to aeroallergens and the early-life origins of allergic disease

Charles Blackley, a family general practitioner in Manchester, first described the characteristic symptoms of hay fever (*Catarrhus Aestivus*).[34] By experi-

menting on himself, Blackley was able to show that hay fever and associated symptoms of asthma followed closely the seasonal appearance of grass and other pollens in the air. Henry Hyde-Salter, a physician and later Dean of Charing Cross Hospital in London, wrote a scholarly Treatise on Asthma in 1860 which drew attention to emanations in dusts as a precipitant of asthma.[35] However, it was not until 1967 that Voorhorst first described the domestic house dust mite (*D. pteronyssinus*) as a major source of allergenic material. Rather than the mite itself being the major source of allergens, it is the faecal particles that contain the highest concentrations of the allergenic digestive enzymes including the cysteine protease *Der* P_1. To survive and reproduce, the house dust mite requires a temperature of 25 °C and a relative humidity of 80%. The adults feed on the proteins present in human skin scales, while the nymphs require hyphae from the fungus *Aspergillus*. In modern housing, these conditions are optimally achieved in the bedding and pillows as well as in carpets, soft furniture and children's soft toys.[36] The faecal particles of the mite contain the highest concentration of allergens. It has been estimated that up to 15% of the contents of vacuum cleaner dust is made up of mites, their body parts and excrement. Contrary to popular opinion, pillows containing artificial fillings are just as good as feather pillows as habitats for dust mites, or even better. Although various chemical agents that kill dust mites (acaricides) have been developed, the only sure way to kill dust mites is to wash materials at a minimum of 60 °C, or freeze them to –20 °C.

Sensitization to dust mites and other domestic allergens most likely occurs early in life. In a prospective study of 17 children in Poole, Dorset, the level of dust-mite allergen present in the home during the first year of life is a major factor in determining whether an infant born of an allergic mother, and therefore genetically at risk of developing allergy or asthma, did in fact do so by the time they reached 11 years of age.[37] The level of allergen per gram of dust was also an important factor in determining the age of onset of first symptoms: the higher the exposure, the earlier the disease onset. Although some of the children who were to develop asthma acquired positive skin tests to dust mites before the age of 5 years, the majority did so between the ages of 5 and 11 years. In children, positive skin-prick tests to mite and cat allergens are the strongest known risk factors for developing asthma.[31,38] In our Dorset study,[37] children who had a positive skin test to mite allergens had a 14.6-fold greater chance of having asthma at age 11 years, compared to those who were skin-test negative.

In a prospective study carried out in the dry climate of Arizona, where sensitization to the fungus *Alternaria* seems to be the major driving allergen source for asthma, maternal, but not paternal, allergy imparted a substantially greater influence on the child's development of elevated levels of serum total IgE and the later development of asthma.[39] While it has been suggested that children may preferentially acquire certain genes from the mother (genomic imprinting), this is very uncommon, and a more likely explanation for the strong maternal influence on the child's phenotype is the influence of the intrauterine environment.[40] It is now well established that intrauterine nutrition is an important factor in programming a child for the later development of such adult degenerative diseases as diabetes, hypertension, chronic obstructive pulmonary disease and osteoporosis.[41] In two separate studies (one retrospective in adults and one prospective in 11-year-old children) we have found a positive relationship between greater head circumference at birth and the later development of allergy and high serum IgE levels.[42] At first such an association may sound strange, but those placental and nutritional factors (especially over nutrition) that increase brain growth in the last trimester of pregnancy may well influence the maturation of the thymus gland, the site of origin of the immune system. It has been suggested that after 26 weeks gestation, the fetus adopts a TH2-like immune phenotype to prevent maternal rejection, and that in the last trimester of pregnancy, with increased IFN-γ production, this converts to a more TH1 picture.[43] Interleukin-4 is produced by the human amnion epithelium throughout pregnancy,[44] and recently IL-10, the cytokine that inhibits TH1 responses, was found in human placenta.[45] If the TH2 cytokine mode is maintained on account of placental factors, then an allergic diathesis might be expected to occur.[46] Such a mechanism might also be invoked as a factor in the causation of some cases of the Sudden Infant Death Syndrome (SIDS, cot death) in which mast cell tryptase[47] and eosinophils[48] are encountered in the lung and circulation.

In babies born to allergic mothers, T lymphocytes removed from the cord blood exhibit enhanced proliferative responses to environmental allergens, such as egg protein (ovalbumin) and milk protein (β-lactoglobulin), as well as those derived from house dust mite, cats and birch pollen.[49] Additionally, asthma and other allergic diseases appear to be more common in those whose mothers were exposed to high concentrations of sensitizing aeroallergens, e.g. birch pollen, during the last two trimesters of pregnancy.[46] How minute amounts of allergen taken in by the mother can cross the placenta to sensitize the offspring is an intriguing but unresolved question. It is possible that small amounts of antigen are trapped by the placenta and are presented to the fetus' immune response at this site or alternatively, that

maternal cells (possibly dendritic cells or macrophages) pass small amounts of antigen through the placenta into the fetal circulation, so that antigen presentation occurs in fetal tissues. A number of groups have shown that at birth those children who go on to develop allergic diseases, including asthma, have impaired cord-blood T-lymphocyte production of interferon-γ (IFN-γ) when their cells are exposed to specific allergen[50,51] (Figure 7). This suggests the existence of an impaired inhibitory mechanism for shutting down a TH2 response rather than one that primarily enhances it. Between the second and third trimesters of pregnancy, the T cells from the fetus spontaneously release IFN-γ, but there are some whose T cells do not release this TH1 cytokine even when stimulated. It has been hypothesized that the role of this fetal IFN-γ production by circulating mononuclear cells is to counteract the effects of IL-4 and IL-10 produced by the placenta, with IFN-γ levels reaching maximum at the time of parturition.[46] A mechanism such as this would be needed to prevent an allergic TH2 phenotype from developing in all newborn children, and its failure may underlie the development of allergy. Environmental factors in pregnancy may also direct the placental-fetal relationship towards a sustained TH2-like response, including young maternal age and smoking in pregnancy.[52]

Viruses and the development of allergic diseases

It has long been recognized that viral infections, especially those of the common cold viruses, can lead to deterioration of asthma lasting for several weeks. It is also known that virus infection in early infancy is responsible for intermittent wheezing illness, and is most frequently caused by the respiratory syncitial virus (RSV). There are thus growing suspicions that virus infections are intimately involved in the development of the asthma syndrome and possibly other manifestations of allergic disease. The application of mRNA detection techniques, such as the polymerase chain reaction, to viruses has paved the way for clarifying any link between viral infections and asthma.[53] For example, in excess of 80% of acute exacerbations of asthma in school children and ~60% in adults are the result of virus infections, the most frequently detected being the common cold viruses (rhinovirus).[54] Mechanisms that may increase the susceptibility of the asthmatic airway to virus-driven inflammation are currently being pursued. One attractive possibility is that in the presence of persistent allergic mast-cell and eosinophil-driven inflammation, the release of certain cytokines, specifically TNFα derived from these cells, leads to an increase in the expression of receptors for human respiratory viruses on the airway-lining epithelium. In the case of the major subtypes of rhinoviruses, the receptor is ICAM-1, which is also involved in leukocyte recruitment from the circulation and in cell–cell signalling (Figure 3).[8] However, ICAM-1 contains an amino-acid sequence that binds strongly to the cavern region of the rhinovirus capsid and enables virus internalization.[55] Once the virus has entered the epithelial cell, it replicates and also generates a range of chemokines (IL-8, RANTES, MCP-1 and eotaxin) which are able to enhance eosinophil- and neutrophil-mediated inflammation.[56]

While viruses can undoubtedly cause deterioration of established asthma, paradoxically, during the first 3 years of life there is evidence to indicate that viral or bacterial infection may protect against the development of allergic disease such as hay fever.[57] One of the most consistent risk factors for allergy in children and adults is family size. In studies carried out both in Germany and in UK birth cohorts, the prevalence of mucosal allergy and positive allergy skin test in children have been shown to decline markedly in the last-born child with increasing numbers of siblings.[58] Over the past 30 years, opportunities for acquiring infections from siblings or playmates in early childhood have declined, with reduction in the average family size, vaccination programmes and higher standards of personal hygiene.[57] Most viruses and some bacteria are able to evoke a TH1-like cell-mediated protective response with the generation of IL-12 and IFN-γ[30] (Figures 7 and 8). Thus, if there are multiple infections during the first few years of life, high concentrations of these TH1 cytokines could inhibit the release of TH2 cytokines, thereby biasing the mucosal immune response airway from allergen sensitization.[59] There is some direct evidence to support such an hypothesis. Shaheen and co-workers, have shown that adolescents aged 13–21 years in Guinea-Bissau, Africa, although infected with measles in the first year of life, when compared to those vaccinated against measles later, had a 63% lesser chance of developing positive skin test to common aeroallergens.[60] It has also been reported that strongly positive tuberculin tests, indicative of exposure to *M. tuberculosis*, predict less asthma, lower IgE levels and predominant TH1 cytokine profiles in Japanese children.[61] Both measles virus and BCG are potent stimulators of the TH1 cytokine response. If given to animals at the same time as a sensitizing antigen, both interleukin-12 and IFN-γ are powerful endogenous agents capable of inhibiting subsequent IgE, mast cell and eosinophilic responses.[30] Thus, it is possible that interleukin-12, IFN-γ or vaccines that are able to preferentially enhance production of these cytokines (e.g. *Mycobacterium vaccae*),[62] may form the

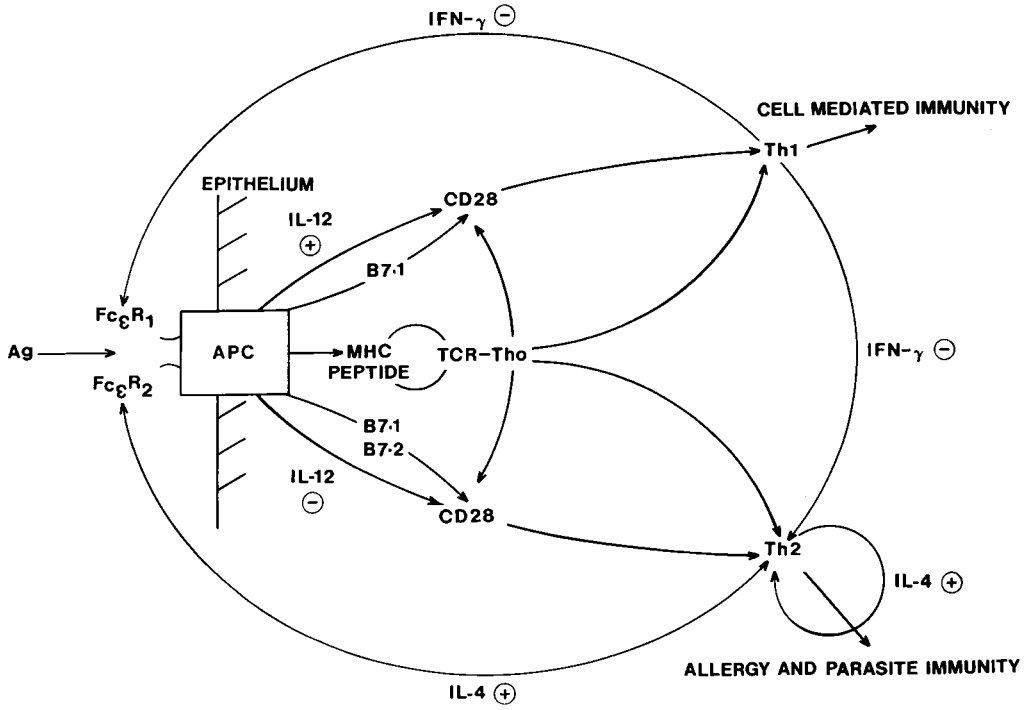

Figure 8. The sequence of events that are thought to lead to differentiation of naive T cells (ThO) to either the Th-1 or the Th-2 phenotypes. Allergen taken up by dendritic cells (APC) by a process which is enhanced by both $Fc_{\epsilon}R1$ and $Fc_{\epsilon}R2$. After processing, the allergen peptide is presented by MHC Class II to the T cell receptor (CD3). Additional engagement of the co-stimulatory molecules CD28 on the T cell and B7 on the APC, together with the presence or absence of IL-12, leads towards the Th-1 or Th-2 subtype. Additional cytokine feedback loops are shown. IL-4 is required for maintenance of a Th-2 response, where IFN-γ encourages a Th-1 response.

basis of new preventative or therapeutic strategies for allergy and asthma.

Changing world-wide trends in asthma and allergy

Epidemiological studies that have used similar methodologies 10–20 years apart have revealed some quite remarkable statistics both in the developed and developing world, pointing towards rising trends in allergic diseases, including asthma (Figure 9). While

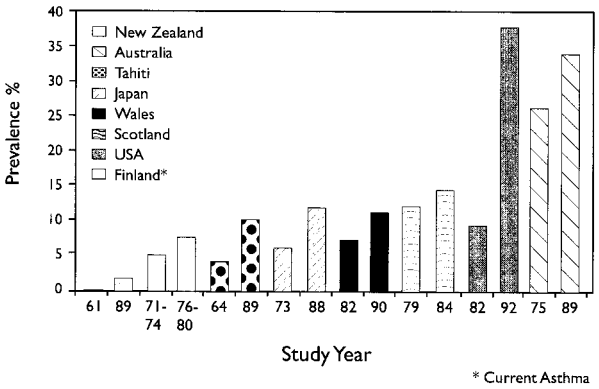

Figure 9. Rising trends in asthma in different countries, using identical methodologies on the two occasions.

it is recognized that these disorders have an important genetic component, it is only in isolated populations that genetic inbreeding could contribute to a progressive increase in asthma and allergy. One example is the high prevalence of asthma in islanders of Tristan da Cunha, all of whom originate from 15 settlers from Scotland, England, N. America, Holland, Italy, St Helen, Ireland and South Africa.[63] However, in outbred populations, changes to our environment are much more likely to be the causes of these rising trends.

The developing world

There are convincing studies in Africa, South America and SE Asia showing substantial increases in the prevalence of asthma associated with population shifts from the rural to the urban environment. In 1975, Godfrey investigated the occurrence of allergy and asthma in Gambian school children, and showed their association with urban dwelling, higher socioeconomic status and lower total circulating IgE levels.[64] He suggested that in the rural setting, parasite infection was protective against the development of allergy and asthma (Figure 10).

Immune defence against invading parasites uses the same components as in allergic tissue responses: IgE, mast cells and eosinophils orchestrated by TH2-

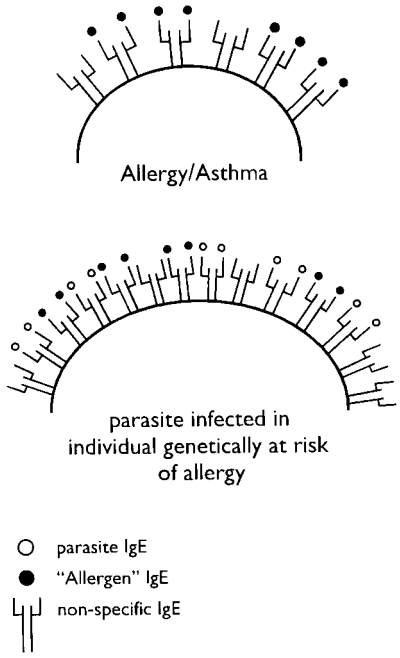

Figure 10. The effects of parasite-directed IgE in reducing mast cell responsiveness to allergens by 'diluting' allergen-specific IgE bound to their receptors on the cell surface.

like lymphocytes.[25] Because parasitic worms in the gut lumen are too large to be destroyed by conventional white blood cell phagocytic mechanisms, the production of parasite-directed IgE, sensitization of mast cells and recruitment of eosinophils leads to the release of mediators of immediate hypersensitivity that cause mucosal events designed to expel the parasite. The anti-parasite response in the gastrointestinal tract is almost identical to that of asthma and rhinitis, comprising excess mucus hypersecretion, contraction of smooth muscle, microvascular leakage and epithelial damage. In asthma and rhinitis, the immune response in the respiratory tract is directed against aeroallergens rather than parasite antigens in the digestive tract.

To further investigate the relationship between parasite infection and the development of allergy, Lynch and co-workers determined the effect of anti-helminthic treatment on the allergic reactivity of children in a slum area of Caracas, Venezuela.[65] The children were divided into two groups, the first being treated for a period of 22 months with the anti-helminth drug Quantrel directed to eliminate the intestinal worms *Ascaris* and *Trichuris*, and a second group who declined treatment and were used as controls. The active treatment almost eliminated the worms (from 68% to 5% in children) and resulted in a decrease in the elevated total serum IgE level (from 2543 to 1124 iu/ml) but, in contrast, was accompanied by an *increase* in skin test reactivity to the house dust mite (from 17 to 68%). In the untreated group, over the 22 month observation period, parasite colonization further increased (from 43 to 70% of children), IgE levels in the serum continued to increase (1649 to 3697 iu/ml) but dust mite sensitization fell (26% to 16%). Application of the PK test and analysis of specific IgE antibody levels indicated that the polyclonal stimulation of IgE synthesis by the parasites resulted in mast-cell receptor saturation and suppression of specific IgE antibody synthesis. Thus, from a public health standpoint, high levels of non-specific IgE may protect rural dwellers exposed to parasites from allergy and asthma. It follows that eradication of parasites or reduced opportunities for infection could in part explain the rural to urban gradient in the prevalence of allergic disease.

The developed world: socioeconomic factors

Population-based surveys in the developed world indicate that the more affluent sections of the community have the highest prevalence of allergic sensitization and associated diseases.[57] Of considerable interest have been the recently reported findings of Von Mutius that, while chronic bronchitis was much more common in former East Germany, asthma, hay fever, and especially allergic sensitization assessed by skin testing, was up to three-fold more prevalent in West Germany.[66] One reason for undertaking these studies was to investigate the influence of industrial-and vehicle-related outdoor air pollution (particulates, NO_2 and SO_2) on airway disease, since this was far worse in former Eastern Europe, as in Western Europe government legislation controllling industrial and domestic emissions resulted in improved air quality. Similar findings have been reported comparing the prevalence of asthma and allergy in the Baltic States with that in Northern Europe. In a further study looking at the influence of age on the apparent East/West difference in the prevalence of clinical allergy and sensitization, the difference was most apparent in children and young adults born since the 1960s.[67] This finding has been interpreted as a cohort effect reflecting economic and cultural differences that followed separation of the two halves of the country following the Second World War. In young British adults, self-reported hay fever and allergy skin-prick tests are significantly related to socioeconomic class as assessed by the father's occupation, the disorders progressively increasing from Social Class IV/V to Social Class I/II[57] (Table 1). One explanation for the effect of socioeconomic factors on the development of allergy is an effect of early maternal programming on allergic sensitization involving dietary, smoking and other factors.

In Australia and in other developed countries,

Table 1 Prevalence (%) of hay fever according to social class and size of family

Father's social class at birth	Number of other children in the household at age 11			
	0	1	2	3+
I/II	26.1	23.1	18.6	17.4
III	19.0	19.6	17.3	11.0
IV/V	21.0	14.7	15.3	9.7
Total	20.6	19.5	17.2	11.2

Adapted from reference 57, with permission.

changes to housing may have produced an important impact. Increased emphasis on energy conservation has resulted in the 'sealing' of homes with tightly fitted windows and doors and, as a consequence, poor air exchange. In older housing, burning of fossil fuels in open fireplaces, heating of rooms only when they were being used and an emphasis on 'airing' of rooms may have kept dust mites and other indoor allergens at a low level. One study of housing in Wagga Wagga, SE Australia, where asthma and allergy prevalence has almost doubled over 10 years, has revealed a 15-fold increase in mite colonization.[68] Increased use of central heating and air conditioning, as well as soft furnishings and carpets associated with improved socioeconomic status, also improves the habitat for dust mites.

A hypothesis advanced to explain both the past and present epidemiological patterns of clinical allergy is that sensitization might be prevented by infections acquired during early childhood. Thus, in East Germany, in the Baltic States, but also in poorer Western civilizations, lower material standards of living and widespread use of day nurseries from an early age with increased spread of infection, might explain the lower prevalence of allergy in these communities. Linking this with an immunological hypothesis, it is tempting to speculate that reduced childhood infections tilts the mucosal immune response away from a TH1-like to a TH2-like response with the subsequent development of the allergic phenotype (Figure 8).

Conclusions

It is most unlikely that the rising trends of allergy and asthma seen world-wide have a single cause. Environmental factors are clearly important, with the increased prevalence of asthma being largely accounted for by increased expression of IgE-dependent hypersensitivity. In the developing countries, the rural-to-urban increase in these disorders can in part be accounted for by changes in parasite infection and increased IgE sensitization to common environmental allergens. In the poorer urban developments, increase in the allergen load, e.g. dust mites encouraged by the uptake of Westernized furnishing and bedding, has increased the population risk of allergen sensitization. In both developing and developed countries, environmental factors operating in pregnancy and in the first 3 years of life are most relevant to the observed increase in asthma and allergy. High socioeconomic status, possibly operating at the fetal-maternal level, increased exposure to indoor allergens and cigarette smoking are all important factors. In drawing together changes to our environment with the early life development of allergy in asthma, a particularly plausible hypothesis is the 'lazy immune or hygiene hypothesis', i.e. that asthma and allergy are epidemics resulting from the absence of infection.[69] Reduced exposure of small children to bacterial and infectious agents, by reducing the TH1-like cytokine influence on the development of the immune response at a time when infants are exposed to high concentrations of allergens, provides a particularly attractive hypothesis for explaining the world-wide trends (Figure 10). If this does turn out to be the case, then a vaccine strategy to enhance the production of a TH1 signal in genetically at-risk children during a 'window of opportunity' seems attractive.

The introduction and implementation of guidelines for asthma management with emphasis on patient education and the effective use of anti-inflammatory drugs ('preventors') rather than relying on bronchodilators ('relievers') for symptom relief alone has resulted in a dramatic reduction in asthma mortality in such countries as New Zealand and the UK. Thus, while understanding of the underlying mechanisms of allergic disease creates new therapeutic opportunities, it is clearly of importance that more effort is made to identify and subsequently remove those environmental factors important in the aetiology of allergic disease.

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References

1. Gell PGH, Coombs RRA, eds. *Clinical Aspects of Immunology*. Oxford, Blackwell, 1964.
2. Prausnitz C, Küstner H. Studien Über Überempfindlichkeit. *Central Bakterol* 1921; **86**:160.

3. Ishizaka K, Ishizaka T. Identification of gamma-E antibodies as a carrier of reagenic activity. *J Immunol* 1967; **99**:1187–92.
4. Helm BA, Sayers I, Higginbottom T, Machado DC, Ling Y, Ahmed K, Padlan EA, Wilson PM. Identification of the high-affinity receptor binding region of immunoglobin E. *J Biol Chem* 1996; **271**:7494–500.
5. Austen KF. From slow-reacting substance of anaphylaxis to leukotriene C_4 synthase. The Paul Kalos Memorial Lecture. *Int Arch Allergy Immunol* 1995; **107**:19–24.
6. Kellaway DH, Trethewie ER. The liberation of a slow-reacting smooth-muscle stimulating substance in anaphylaxis. *Q J Exp Physiol* 1940; **30**:121–45.
7. Seminario M-C, Gleich GJ. The role of eosinophils in the pathogenesis of asthma. *Curr Opin Immunol* 1994; **6**:860–4.
8. Bochner BS, Luscinskas FW, Grimbrone MA Jr, Newman W, Sterbinsky SA, Derse-Anthony C-P, Klunk D, Schleimer RP. Adhesion of human basophils, eosinophils and neutrophils to interleukin-1 activated human vascular endothelial cells: contribution of endothelial cell adhesion molecule. *J Exp Med* 1991; **173**:1553–6.
9. Montefort S, Holgate ST. Expression of cell adhesion molecules in asthma. In: Bochner BS, ed. *Adhesion molecules in allergic disease*. New York, Marcel Dekker, 1997:315–38.
10. Kita H. Regulation of eosinophil mediator release by adhesion molecules. In: Bochner BS, ed. *Adhesion molecules in allergic disease*. New York, Marcel Dekker, 1997:227–55.
11. Corrigan CJ, Kay AB. The lymphocyte in asthma. In: Busse WW, Holgate ST, eds. *Asthma and Rhinitis*. Boston, Blackwell Scientific Publications, 1995:450–64.
12. Semper AE, Hartley JA. Dendritic cells in the lung: What is their relevance to asthma? *Clin Exp Allergy* 1996; **26**:485–90.
13. Bellini A, Vittori E, Marini M, Ackerman V, Mattoli S. Intraepithelial dentritic cells and selective activation of Th2-like lymphocytes in patients with atopic asthma. *Chest* 1993; **103**:997–1005.
14. Kelso A. Th1 and Th2 subsets: paradigms lost? *Immunology Today* 1996; **16**:374–9.
15. Aversa G, Pannonen J, Cocks BG, Waal Mallefyt RD, Vega F, Zurawski SM, De Vries JE. An Interleukin 4 (IL-4) mutant protein inhibits both IL-4 or IL-13 induced human immunoglobulin G4 (IgG4) and IgE synthesis and B cell proliferation: support for a common component shared by IL-4 and IL-13 receptors. *J Exp Med* 1993; **178**:2213–17.
16. Sutton BJ, Gould HJ. The human IgE network. *Nature* 1993; **366**:42108.
17. Hewitt C, Brown A, Hart B, Prichard D. A major house dust mite allergen disrupts the immunoglobulin network by selectively cleaving CD23: Innate protection by anti-proteases. *J Exp Med* 1995; **182**:1–8.
18. Coyle A, Wagner K, Bertrand C, Touyaki S, Bews J, Heusser C. Central role of immunoglobulin (Ig) E in the induction of lung eosinophil infiltration and T helper 2 cytokine production: Inhibition by a non amphylactogenic anti-IgE antibody. *J Exp Med* 1996; **183**:1303–10.
19. Saban R, Haak-Frednscho M, Zine M, Ridgeway J, Gorman C, Presta LG, Bjorling D, Saban M, Jordieu P. Human $Fc_\epsilon R1$-IgG and humanised anti-IgE monoclonal antibody MaE11 block passive sensitisation of human and rhesus monkey lung. *J Allergy Clin Immunol* 1994; **94**:836–43.
20. Corne J, Djukanović R, Thomas L, Warner J, Botta L, Grandordy B, Gygax D, Heusser C, Patalano F, Richardson W, Kilchherr E, Staehelin T, Davis F, Gordon W, Sun L, Louis R, Wang G, Chang T-W, Holgate ST. The effect of intravenous administration of a chimaeric anti-IgE antibody on serum levels in atopic subjects: Efficacy, safety and pharmacokinets. *J Clin Invest* 1997; **99**:829–37.
21. Boulet L-P, Chapman KR, Côté J, Kalra S, Bhagat R, Swystun VA, Laviolette M, Cleland LD, Deschesnes F, Su JQ, DeVault A, Fick Jr RB, Cockcroft DW. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. *Am J Respir Crit Care Med* 1997; **155**:1835–40.
22. Fahy JV, Fleming HE, Wong HH, Liu JT, Su JQ, Reimann J, Fick Jr RB, Boushey HA. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am J Respir Crit Care Med* 1997; **155**:1828–34.
23. Shakib F, Smith SJ. *In vitro* basophil histamine-releasing activity of circulating IgG 1 and IgG4 autoanti-IgE antibodies from asthma patients and the demonstration that anti-IgE modulates allergen induced basophil activation. *Clin Exp Allergy* 1994; **24**:270–5.
24. Moll H. Immune responses to parasites: the art of distinguishing the good from the bad. *Immunology Today* 1996; **17**:551–2.
25. Kojima S. Eosinophils in parastic diseases. In Makino S, Fukuda T, eds. *Eosinophils: Biological and Clinical Aspects*. Tokyo, CRC Press, 1993:391–402.
26. Stewart GA, Thompson PJ. The biochemistry of common aeroallergens. *Clin Exp Allergy* 1996; **26**:1020–44.
27. Stewart GA, Kollinger MR, King CM, Thompson PJ. A comparative study of three serine proteases from *Dermatophagoides pteronyssimus* and *D. farinae*. *Allergy* 1994; **49**:553–60.
28. Howell WM, Holgate ST. Human leukocyte antigen genes and allergic disease. In: Hall IP, ed. *Genetics of Asthma and Atopy*, Basel, Karger, 1996:53–70.
29. Rosenwasser LJ, Klemm DJ, Dresback JK, Inamura H, Mascali JJ, Klinnert M, Borish L. Promoter polymorphisms in chromosome 5 gene cluster in asthma and atopy. *Clin Exp Allergy* 1995; **25**(Suppl.2):74–8.
30. Scott P. IL-2: Initiation cytokine for cell mediated immunity. *Science* 1993; **206**:2496.
31. Peat JK, Woolcock AJ. Sensitivity to common allergens: relation to respiratory symptoms and bronchial hyper-responsiveness in children from three different climatic areas in Australia. *Clin Exp Allergy* 1991; **21**:573–81.
32. Chapman MD. Cockroach allergens: a common cause of asthma in North American cities. *Insights Allergy* 1993; **8**:1–8.
33. Burrows B, Martinez FD, Halonnen M, Burbee RA, Clin MG. Association of asthma with serum IgE levels and skin test reactivity to allergens. *N Engl J Med* 1989; **320**:271–7.
34. Blackley CH. Experimental researches on the causes and nature of *Catarrhus Aestivus* (Hay Fever or Hay Asthma). London, Balliére, Tindall and Cox, 1873.
35. Salter HH. *On Asthma: Its Pathology and Treatment,* 1st edn. London, Churchill, 1860.
36. Siebers RW, Fitzharris P, Crane J. Beds, bedrooms and bugs: anything new between the sheets? *Clin Exp Allergy* 1996; **26**:1225–7.
37. Sporik R, Holgate ST, Platts-Mills TAE, Coggswell JJ.

Exposure to house dust mite allergen ($DerP_1$) and the development of asthma in childhood. A prospective study. *N Engl J Med* 1990; **323**:502–7.

38. Burney PGJ. Current questions in the epidemiology of asthma. In: Holgate ST, Austen KF, Lichtenstein LM, Kay AB, eds. *Asthma: Physiology, Immunopharmacology and Treatment*. London, Academic Press, 1993:3–16.
39. Martinez FD, Holberg CJ, Halonen M, Morgan WJ, Wright AL, Taussig LM. Evidence of mendelian inheritance of serum IgE levels in hispanic and non-hispanic white families. *Am J Hum Genet* 1994; **55**:555–65.
40. Doull IJM. The maternal inheritance of atopy. *Clin Exp Allergy* 1996; **26**:613–15.
41. Barker DJP, ed. *Fetal and infant origins of adult disease*. London, British Medical Journal, 1992.
42. Godfrey KM, Barker DJP, Osmond C. Disproportionate fetal growth and raised IgE concentration in adult life. *Clin Exp Allergy* 1994; **24**:641–8.
43. Warner JA, Jones AC, Miles EA, Colwell BM, Warner JO. Maternofetal Interaction and allergy. *Allergy* 1996; **51**:447–51.
44. de Moraes-Pinto MI, Vince GJ, Flanagan BF, Hart CA, Johnson PM. Localisation of IL-4 and IL-4 receptors in the human term placenta, decidua and amniochorionic membranes. *Immunology* 1997; **90**:87–94.
45. Cadet P, Rady PL, Tyring SK, Yandell RB, Hughes TK. Interleukin-10 messenger ribonucleic acid in human placenta: implications of a role for interleukin-10 in fetal allograft protection. *Am J Obstet Gynecol* 1995; **173**:25–9.
46. Wegmann TG, Lin H, Guilbert L, Mossman TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th-2 phenomenon? *Immunology Today* 1993; **14**:353–6.
47. Holgate ST, Walters C, Walls AF, Lawrence S, Shell DJ, Variend S, Fleming PJ, Berry PJ, Gilbert RE, Robinson C. The anaphylaxis hypothesis of sudden infant death syndrome (SIDS): mast cell degranulation in cot death revealed by elevated concentrations of tryptase in serum. *Clin Exp Allergy* 1994; **14**:1115–22.
48. Howat WJ, Moore IE, Judd M, Roche WR. Pulmonary immunopathology of sudden infant death syndrome. *Lancet* 1994; **343**:1390–2.
49. Miles EA, Warner JA, Jones AC, Colwell BM, Bryant TN, Warner JO. Peripheral blood mononuclear cell proliferative responses in the first year of life in babies born of allergic parents. *Clin Exp Allergy* 1996; **26**:780–8.
50. Warner JA, Miles EA, Jones AC, Quint DJ, Colwell BM, Warner JO. Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema. *Clin Exp Allergy* 1994; **24**:223–30.
51. Tang MLK, Kemp AS, Thorburn J, Hill DJ. Reduced interferon-γ and subsequent atopy. *Lancet* 1994; **344**:983–5.
52. Martinez FD, Clive M, Burrows B. Increased incidence of asthma in children of smoking mothers. *Paediatrics* 1992; **89**:21–6.
53. Johnston SL, Sanderson G, Pattemore PK, Smith S, Bardin PG, Bruce CB, Lambden PR, Tyrell DA, Holgate ST. Use of polymerase chain reaction for diagnosis of picornovirus infection in subjects with and without respiratory symptoms. *J Clin Microbiol* 1993; **31**:111–17.
54. Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, Symington P, O'Toole S, Myint SH, Tyrell DAJ, Holgate ST. Community study of role of viral infections in exacerbations of asthma in school children in the community. *Br Med J* 1995; **310**:1225–9.
55. Ohlin A, Hoover-Litty H, Sanderson G, Paessens A, Johnston SL, Holgate ST, Huguenel E, Greve JM. Spectrum of activity of soluble intercellular adhesion molecule 1 against rhinovirus reference strains and field isolates. *Antimicrobial Agents and Chemotherapy* 1994; **38**:1413–15.
56. Sabuste MC, Jacoby DB, Richards SM, Proud D. Infection of a human respiratory cell line with rhinovirus. Induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. *J Clin Invest* 1995; **96**:549–57.
57. Strachan D. Socioeconomic factors and the development of allergy. *Toxicol Letts* 1996; **86**:199–203.
58. von Mutius E, Martinez FD, Fritzsch C, Nicolai T, Reitman P, Thiemann HH. Skin test reactivity and number of siblings. *Br Med J* 1994; **308**:692–6.
59. Martinez FO. Role of viral infections in the inception of asthma and allergies during childhod: could they be 'protective'? *Thorax* 1994; **49**:1189–91.
60. Shaheen SO, Aaby P, Hall AJ, Barker DJP, Heyes CB, Sheill AW, Goudiaby A. Measles and atopy in Guinea-Bissau. *Lancet* 1996; **347**:1792–6.
61. Hopkin JM, Enomoto T, Shimazu S, Shirakawa T. Inverse association between tuberculin responses and atopic disorder. *Science* 1997; **275**:77–9.
62. Vacirca A, Dominino J, Valentini E, Hartopp R, Bottasso OA. Pilot study of immunotherapy with *M. vaccae* against tuberculosis. *Tubercle Lung Dis* 1994; **75**:47–8.
63. Zamel N, McClean PA, Sandell PR, Siminovitch KA, Slutsky AS and the University of Toronto Genetics of Asthma Research Group. Asthma on Tristan da Cunha: Looking for a genetic link. *Am J Respir Crit Care Med* 1996; **153**:1902–6.
64. Godfrey RC. Asthma and AgE levels in rural and urban communities of the Gambia. *Clin Allergy* 1975; **5**:201–7.
65. Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R, Alvarez N. Effect of antihelminitic treatment on the allergic reactivity of children in a tropical slum. *J Allergy Clin Immunol* 1993; **92**:404–11.
66. von Mutius E, Martinez FD, Fritzch C, Nicolai T, Röll G, Thiemann H. Prevalence of asthma and atopy in two areas of West and East Germany. *Am J Respir Crit Care Med* 1994; **149**:358–64.
67. Weichmann HE. Environment, lifestyle and allergy: the German answer. *Allergol* 1995; **4**:315–16.
68. Peat J, Tovey E, Toelle BG, Haby MM, Gray EJ, Mahmil A, Woolcock AJ. House dust mite allergens. A major risk factor for childhood asthma in Australia. *Am J Respir Crit Care Med* 1996; **153**:141–6.
69. Cookson WOCM, Moffatt MF. Asthma: An epidemic in the absence of infection. *Science* 1997; **275**:41–2.