CURRENT TOPIC

Molecular biology and genetics of allergy and asthma

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In the past few years considerable progress has been made in understanding the pathogenesis of allergy at the cellular and subcellular level. In particular, advances in cell biology and DNA technology have shed light on the cell interactions that are pivotal in orchestrating the inflammation underlying allergy. Although much has now been established in the cellular mechanism of allergic inflammation, the fundamental question of why some people suffer from the disease and others do not has only begun to be answered by molecular genetics, which has implicated the inheritance of several genes that predispose to the development of allergy.

Definitions

Allergy comprises a group of syndromes that includes asthma, atopic dermatitis, and hay fever, and tends to occur in familial clusters. These diseases have classically been described as caused by an allergic response characterised by immediate hypersensitivity reactions (that is, weal and flare to intradermal allergens), increased serum IgE, and increased bronchial reactivity to specific or non-specific inhaled allergens. In contrast, a significant proportion of patients with asthma and urticaria are seen without an atopic background. This is limited to the molecular biology of atopic asthma as an example to illustrate the advances in the basic science of allergy.

Syndrome of asthma

Asthma is a syndrome characterised by diffuse narrowing of the bronchi that is relieved either spontaneously or after appropriate treatment with bronchodilators or anti-inflammatory drugs. Asthma is a spectrum of diseases ranging from paroxysms of cough, wheeze, and dyspnoea occurring periodically over time with complete or near complete symptom free periods, to persistent chronic asthma with frequent symptoms and the need for continuing treatment to maintain control of symptoms, and asthma that is resistant to steroids and is characterised by an incomplete reversibility of airflow obstruction despite treatment with bronchodilators and corticosteroids in high doses.

Prevalence

Asthma affects about 10% of children and 5% of adults. Current estimates indicate that 3.2 million adults in the UK are affected. This disease causes considerable mortality and morbidity, with 2000 potentially avoidable deaths each year. The mainstay of treatment is inhaled corticosteroids. Although mild asthma can be safely treated with low doses of inhaled corticosteroids, patients with moderate and severe asthma are often receiving high doses of inhaled or systemic steroids, or both. The long term consequences of steroids given by mouth are well documented, with side effects such as osteoporosis, skin thinning, and hypertension.

Pathogenesis

The immune response associated with asthma is characterised by infiltration of the bronchial mucosa with mast cells, eosinophils, macrophages, lymphocytes, and plasma cells.[1–4] Cytokines derived from T helper 2 (T_{H2}) lymphocytes and mast cells are implicated in the pathogenesis of asthma.[3 5] T helper lymphocytes have been shown to produce interleukin 3 (IL)-3, IL-4, IL-5, IL-10, IL-13, and granulocyte macrophage colony stimulating factor (GM-CSF), whereas mast cells produce IL-4, IL-5, IL-6, and tumour necrosis factor α (TNFα).[5–8] The consequences of the release of these cytokines include the maturation and recruitment of mast cells (IL-3, IL-9), eosinophils (IL-4, IL-5), and macrophages (GM-CSF).[9–12] Eosinophils have been implicated as the primary effector cell responsible for the induction of bronchial mucosal injury.[13 14] A further characteristic feature of the asthmatic response is the over expression of IgE from B cells (IL-4, IL-6, IL-13).[15]

Contribution of inherited factors

The central tenet of research in asthma genetics is that clinical disease only occurs in a subject with a genetic susceptibility, which becomes expressed after exposure to an environmental trigger. Several environmental factors have been proposed, including house dust mites, cigarette smoke, viral respiratory tract infections, and atmospheric pollution. Unfortunately, the elements of the genetic susceptibility have proved to be more elusive. Although the prevalence of asthma in a population is approximately 4–8% (using symptom based questionnaires), this increases to 20–25% in those with affected first degree relatives. This measure can be expressed as the

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risk ratio (λ_S), which is defined as the prevalence of a disease in first degree relatives of an affected subject divided by the prevalence in the general population. In asthma, λ_S is approximately 5–6, compared with 15 in type I diabetes mellitus, 8 in schizophrenia, and 3.5 in type II diabetes mellitus.

Monozygotic and dizygotic twin studies have examined the concordance of a number of traits, such as asthma symptoms, total IgE, and skin test hypersensitivity, and have shown variable, but significant, inherited contributions (reviewed elsewhere[16]). As with other multifactorial diseases, the contribution of genetic factors is influenced by the population being studied and the propensity to sharing a common environment.

A number of studies has noted parent of origin effects in the inheritance of atopy. In particular, some, but not all, studies have shown an increased risk of atopy in children whose mother was atopic.[17–22] This maternal effect has also been noted in some of the molecular genetic studies of asthma and atopy.[23 24] The mechanism of this phenomenon remains obscure, although several hypotheses have been advanced, including immunological interactions between the mother and child, genetic imprinting, and bias in the populations.[25]

Characterisation of asthma by eosinophilic inflammation

Histopathological studies of the airway inflammation in asthma have found an increase of eosinophils, lymphocytes, plasma cells, and mast cells in patients with asthma. The resultant phenomena of mucosal oedema, vascular congestion, bronchoconstriction, increased mucus production, and impaired ciliary function are thought to be a consequence of the interaction and activation of these inflammatory cells. In addition, several in vivo studies have shown the presence of allergic inflammation in those affected by persistent asthma as well as asthma induced in susceptible subjects by exogenous antigens. In patients with mild, stable asthma with persistent symptoms and an increased airway responsiveness, bronchial biopsy samples and broncheoavleolar lavage cell studies have shown an increased number of eosinophils, lymphocytes, and mast cells. In addition, the eosinophils showed evidence of activation with increased concentrations of major basic protein, eosinophilic cationic protein, and other granular proteins. Such findings in patients with mild, stable asthma not only provide clues to the pathogenesis, but should also raise considerable concern in doctors as they indicate chronic inflammation in those otherwise considered to have trivial asthma.

T helper (CD4+) lymphocytes

T cells exist as two principal subsets determined by function, which may be discriminated by the specific antibody markers CD4+ and CD8+. The cell of principal interest in allergic inflammation is the CD4+ T cell, which is activated by soluble peptides presented by antigen presenting cells (macrophages, B lymphocytes, and dendritic cells) in combination with major histocompatibility complex (MHC) class II molecules on the cell surface. In contrast, CD8+ cells recognise peptide antigen in combination with MHC class I molecules, reflecting their role in combatting intracellular pathogens by direct cytotoxic action.

The downstream effects of both T cell types are mediated by a family of cytokines. The CD4+ T cells in mice have been shown to exist in two distinct subsets, T_{H1} and T_{H2}, based on differences in cytokine secretion. No surface markers have yet been identified to distinguish between these subsets. The existence of such subsets in humans is the subject of much debate.

T HELPER SUBSETS

Both subsets secrete IL-3, GM-CSF, and TNF. Cells similar to T_{H1} additionally secrete IL-2, TNFβ, and interferon γ (IFNγ), and participate in delayed hypersensitivity reactions. Cells similar to T_{H2} produce IL-4, IL-5, IL-10, and IL-13. The significance of these subsets has been appreciated since the discovery of the importance of the cytokines IL-4 and IL-5 in allergy. IL-4 and IL-13 promote B cell antibody class switching, favouring IgE and IgG_4 production and possibly autocrine feedback promoting the T_{H2} cell line commitment of naive T cells. IL-5 promotes the terminal differentiation of eosinophils and increases longevity through decreased apoptosis.

One of the fundamental issues raised by the appreciation of the existence of T cell subsets and their possible role in allergy was the mechanism by which differentiation down either T_{H1} or T_{H2} was regulated. The role of IL-4 in promoting differentiation along the T_{H2} line has been appreciated for some time, but its source remains obscure. Recent evidence suggests that the dendritic cell is important in the primary activation of the T cell by inducing IL-4 gene expression.

Novel genes in the regulation of IgE/IgG4: proto-oncogene c-maf

Ho *et al* identified the crucial role of c-maf (a proto-oncogene) in the T_H subset specific expression of IL-4.[26] The transcription of cytokine genes is regulated by a number of transactivating factors, including the family of nuclear factors of activated T cells (NF-AT) and auxiliary transcription factors of the AP-1 family. These factors are not responsible for the differential expression of cytokines between T_{H1}/T_{H2} subsets, however, as NF-AT family members bind and transactivate a number of cytokine genes, and there is no evidence of the differential expression of AP-1 family members in these cells. In a series of experiments it was shown that c-maf was responsible for the tissue specific regulation of IL-4 in T_{H2} cells. In addition, it was shown that c-maf was selectively expressed in differentiating and mature T_{H2} cells, but was absent from T_{H1} cells. Furthermore, it has been shown that Shc, an adaptor

protein in the ras MAPKinase pathway, is important in modulation IgG_4 secretion.

Mast cells and inflammatory mediators

The mast cell is activated by dimerisation of antigen specific IgE bound to high or low affinity receptors on the cell surface. A wide variety of chemical mediators is released by the mast cell, such as histamine, bradykinin, leukotrienes C, D, and E, platelet activating factor, PGE_2, $PGF_{2\alpha}$, PGD_2, and thromboxane. In addition, several chemotactic factors are released, including eosinophil and neutrophil chemotactic factor of anaphylaxis and LTB_4. Lung biopsy sample studies suggest that the mast cell is more important in mild atopic asthma, as distinct from severe chronic asthma.

Eosinophils

Eosinophils are probably the key effector cell in producing the tissue damage in asthma. Activation and degranulation of eosinophils are triggered by activation through their IgE, IgA, and IgG surface receptors with or without direct stimulation by mediators such as PAF or LTB_4. The proinflammatory mediators released include eicosanoids such as LTC_4 and LTD_4, PAF, and four basic proteins including major basic protein and eosinophil peroxide, which are thought to be toxic to the respiratory epithelial cells. In addition to the secretion of these mediators, eosinophils also secrete a range of cytokines including IL-1, IL-3, IL-5, IL-6, IL-8, GM-CSF, transforming growth factor α (TGFα), and TGFβ. Of note, GM-CSF has been shown to have an autocrine effect on cells, enhancing cell survival.

The eosinophil is produced in the bone marrow, and differentiation and survival are promoted by the lymphokines IL-3, IL-5, and GM-CSF. IL-5 has the dual distinction of specifically inducing the terminal differentiation of the committed eosinophil precursor and promoting eosinophil survival. In contrast, IL-3 and GM-CSF promote maturation in a number of lymphocyte lines in addition to preventing eosinophil apoptosis.

The accumulation of eosinophils in the airway mucosa of patients with asthma has been attributed to both increased sequestration and increased longevity. Chemokines are a novel family of cytokines with chemoattractant properties, which now contains over 30 members. They appear to have a crucial role in inflammation by acting to increase both the recruitment of specific cells, particularly leukocytes, and their activity.[27 28] Of particular interest are eotaxin and IL-8. Eotaxin preferentially attracts eosinophils in vivo and has increased expression in animal models of allergic inflammation and in human tissue in which there is eosinophil accumulation. These features suggest that eotaxin is an important chemokine in the pathophysiology of allergic disorders. IL-8 has chemoattractant activity for a wide spectrum of leukocyte cell types (neutrophils, T cells, macrophages, eosinophils, and others), in addition to promoting neutrophil adhesion, degranulation, and microbicidal activity. The discovery that IL-5 will specifically enhance the locomotor response of the eosinophil to PAF, LTB_4, and IL-8 has given a potential explanation for their differential sequestration in allergic inflammation. IL-3 and IL-5 have also been shown to enhance eosinophil margination on the endothelial cells of capillaries by the upregulation of adhesion molecules on eosinophils, but not neutrophils. A number of other chemokines have chemoattractant activity for eosinophils, including MCP-4 (monocyte chemoattractant protein) and RANTES (regulated on activation, normal T expressed, and secreted). Other molecules having chemoattractant activity have been described, such as PAF and C5a, which recruit the cells to sites of inflammation. These factors, however, are non-specific and will also recruit neutrophils.

Several lines of research have shown that apoptosis of eosinophils occurs within the airway and that this process leads to their recognition and ingestion by macrophages without inciting tissue injury, unless cytokines such as IL-3, IL-5, and GM-CSF inhibit apoptosis.[29] In contrast, glucocorticoids induce apoptosis, despite the presence of these cytokines.[30] The induction of apoptosis is therefore a critical pathway of the resolution of inflammation in asthma. Apoptosis is a highly organised process by which cells undergo programmed cell death. Genes have been identified that either promote or inhibit the process such as bcl2, Ras, c-abl, and ICE.[31]

Antigen presentation via macrophages

Macrophages collaborate with B cells and T_H cells in the production of antibody. Macrophages will often be the first cell to encounter a foreign protein and will non-specifically engulf such material. Subsequent degradation and processing occurs within the cell, which will then present a fragment of the original protein (9–15 amino acids long) in conjunction with MHC II molecules on its surface to T_H cells via the T cell receptor (TCR) (fig 1). The resulting activation of T_H cells produces two principal effects enhancing antibody production by B cells: directly through the CD40 receptor of the B cell, and by inducing the production of IL-4 by the T_{H2} cell. The B cell also has the molecular capability of endocytosing antigen and presenting antigenic peptides via the MHC class II to effector cells.

B cell immunoglobulin class switching

Antibody production occurs in B lymphocytes and its mature cell type, the plasma cell. The antibody molecule is constructed from two heavy chains and two light chains linked by disulphide bridges. The antibody molecule has two functional domains. The antigen binding specificity is determined by the NH2 terminus of the immunoglobulin heavy and light chains, which are extremely variable. The COOH terminus of the immunoglobulin heavy chain determines the effector functions. The variable regions of both heavy and light chains interact to form the antigen binding domain. The variable amino acid structure of this region is created by a number of mechanisms, including

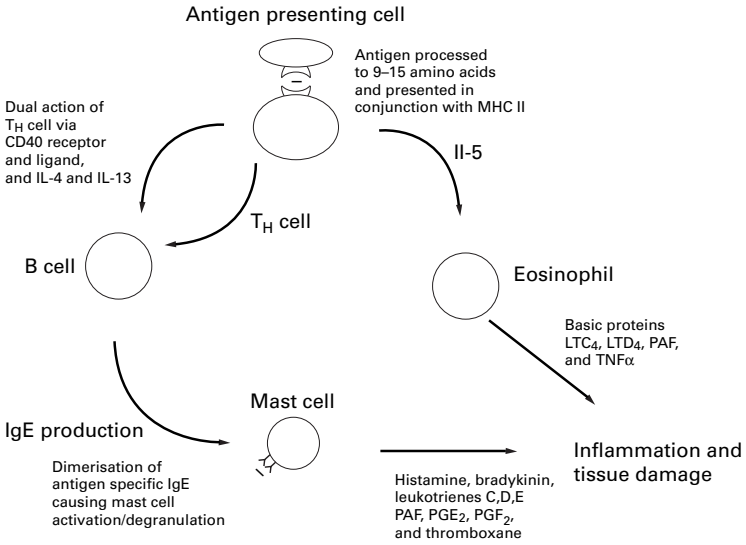

Figure 1 Antigen presentation and subsequent role of T cells in the pathogenesis of asthma and atopy.

DNA rearrangement and somatic recombination to produce the V(D)J domain.

The immune system has evolved an almost inexhaustible versatility in the production of specific antibodies. The accepted model for this phenomenon is the clonal selection theory, which assumes a constant production of B cells with surface immunoglobulin with unique antigen specificity. When a novel antigen is encountered by the immune system, this will bind only to a small fraction of the surface immunoglobulin with the appropriate specificity. The binding of antigen with the surface antibody initiates a cascade stimulating that B cell to proliferate and to synthesise and secrete more specific antibody.

The humoral immune response is generated by the production of specific antibodies to a foreign antigen. B cells can sequentially express different heavy chain isotypes with the same V(D)J region. This ability of heavy chain class switching allows the antibody to retain its antigen specificity, but to change its effector action. This is achieved by a recombination event bringing a new heavy chain gene into continuity with the VDJ gene, with the previously expressed CH gene being deleted.

Class switching and the elaboration of IgE is regulated by lymphokines and the interaction with T cells. IL-4 plays a central part in these events. Several experiments using murine and human B cells have reported that IL-4 is sufficient to direct transcription through the ε locus, but is not effective in inducing significant IgE production.[32] T Cells function in a dual capacity with their direct role as a second stimulus through binding of the T cell CD40 ligand to the B cell CD40 antigen, and as a source of IL-4. In vitro Epstein–Barr virus or direct stimulation of the CD40 antigen are necessary for the production of ε transcripts and IgE synthesis.

Molecular genetics of allergy and asthma

The study of the inheritance of allergy and asthma has been the subject of intense scrutiny, but despite much effort has consistently evaded description in terms of simple Mendelian genetics. There are a number aspects worthy of discussion. The genetic loci of a number of rare single gene disorders have been established. This contrasts with the situation for many common diseases (hypertension, type I diabetes, and schizophrenia) in which familial clustering occurs, suggesting an inherited component in which the genetic mechanisms do not fit a classic genetic model. Several possible reasons have been proposed for this deviation from simple Mendelian genetics: more than one gene in each individual may interact to produce the disease phenotype (polygenic); different genes may exist in different individuals (genetic heterogeneity); and interaction with the environment leads to variable expression and severity.

The first obstacle encountered by those trying to study the genetics of allergy and asthma is the difficulty in defining the disease phenotype. In many studies an attempt to circumvent this has taken the form of using surrogate markers (which often allow objective measures), including the use of total or specific serum IgE concentrations, skin test reactions for hypersensitivity, and bronchial hyperresponsiveness. Reliance on symptom records or the clinical diagnosis of asthma is open to a great number of confounding factors. In addition, the method of selection (ascertainment) of the study population may have significant effects by introducing bias.

PHENOTYPE DEFINITION

Asthma has several intermediate phenotypes that can be studied either as quantitative or qualitative measures. Asthma may be determined by standard symptom questionnaires or by doctor diagnosis based on the demonstration of variable airflow obstruction.[33] In addition, the non-specific bronchial hyperresponsiveness that accompanies asthma may be shown by measuring the dose response curve of airflow against a bronchoconstrictor such as metacholine or histamine. Several studies have found a significant association between bronchial hyperresponsiveness and allergy.[34 35] Atopy manifests itself as an IgE response to inhaled allergens. This may be detected by skin prick tests to allergens, a significant result being a weal greater than the negative control by 3 mm at 10–15 minutes. An alternative measure of atopy is the serum total or specific (RAST) IgE concentrations. An increase in total serum IgE has been shown to correlate with allergy and asthma.[36 37] Eosinophils have also been shown to be increased in peripheral blood, sputum, bronchoalveolar lavage fluid, and bronchial biopsy samples in patients with asthma compared with normal subjects, and increasing levels correlated with increased clinical severity.[38 39]

MOLECULAR GENETIC APPROACHES

Traditionally, finding the gene responsible required the discovery of a biochemical or physiological abnormality leading to the isolation of an aberrant protein that was partially

sequenced. This amino acid sequence was then used to produce an oligonucleotide to screen for the expressed gene, which could then be fully sequenced. In many diseases there is no known abnormal protein and the molecular geneticist has to resort to a number of approaches based on the statistical analysis of the segregation of the disease phenotype with a region of the genome (locus). Once a linkage is established, several approaches are necessary to home in on the aberrant gene. Several loci influencing atopic asthma have been identified previously through two complementary techniques: the study of candidate genes and by genetic linkage and positional cloning.[40]

Positional cloning

In the absence of a known biochemical defect, positional cloning rests with the ability to determine those chromosomal regions that tend to be shared among affected relatives and tend to differ between affected and unaffected relatives. This process involves scanning the genome with closely interspersed genetic markers, determining the linkage statistic, and then identifying the regions with linkage statistics greater than those expected by independent assortment.[41 42] Refinement of the genetic map will be followed by obtaining the DNA clones from this region of interest and searching for differences in the structure of mutant and wild-type DNA, or in the expression of specific mRNAs to identify the gene whose function has been disrupted.

Genetic and physical mapping

Localising a gene of interest often uses two complementary strategies: the establishment of a region of a chromosome that segregates with the disease trait (genetic mapping) and the physical characterisation (mapping) of this region, often in large DNA vectors such as yeast artificial chromosomes. Several approaches have been successfully used to determine the disease locus. Of these, one of the most frequently used in asthma genetics research has been sib pair analysis. Affected sib pair analysis provides the simplest situation, with the environmental factors assumed to have already acted and produced the disease expression, and the advantage that it makes no assumption about the mode of inheritance. This method involves determining whether affected relatives inherit a region more often than expected under independent Mendelian segregation.[43 44] Sib pair analysis has also been applied to quantitative traits and to multipoint linkage mapping. The techniques of physical mapping have been reviewed elsewhere.[45 46]

CANDIDATE GENES

Chromosome 11q13 and the β subunit of the high affinity IgE receptor (FcεRI-β)

Sandford and colleagues[40] reported the presence of two high affinity IgE receptor subunit polymorphisms ile/leu181 and val/leu183 in 155 multiple sibships. An association was shown between leu181 and antigen specific atopic responses.[47] This work has been confirmed in a Japanese population and in two separate Australian populations. These two populations comprised 1000 random subjects from Bussleton in whom the frequency of leu181/183 was 4% and maternally inherited, and 241 subjects from 20 extended atopic Aboriginal families from Kalumburu, in whom the frequency of leu181/183 was 34% and not maternally inherited, but was associated with high total IgE and lower specific IgE and skin test responses (Cookson WOCM, personal communication; Hill M, Cookson WOCM, personal communication).[48]

There are, however, several other studies that did not confirm these data. Hall IP *et al* (personal communication) sequenced the region of leu181/183 in 120 subjects from 40 nuclear families with at least one atopic subject and did not show any polymorphism. In addition, in collaboration with two other groups using ARMS, we have not detected any leu181/183 mutations (unpublished data). Work from the laboratory of Kinet using site directed mutagenesis has show that leu181 mutations have no effect on IgE binding, signal transduction, or receptor assembly. In addition, transgenic knockout mice for the FcεRI-β do not show maternal imprinting of the gene in offspring.[49]

There has been much dispute over the linkage of atopy to chromosome 11q13. The initial studies of Cookson and Hopkin, which showed a dominant gene on chromosome 11q13 using pλMS51, have been reproduced by one group.[50–52] Five studies, however, have not reproduced these results.[53–56 58] Unpublished work from Marsh and colleagues has shown a linkage between another chromosome 11q13 marker and atopy, and data from Hizawa *et al* show an association between high serum total IgE and D11S97.[59] Furthermore, we have excluded an immunomodulatory gene CC10 that localises to chromosome 11q13 as a candidate for this region. This implies that another locus may be present on 11q that predisposes to atopy.

Chromosome 14

Chromosome 14q contains several candidate genes that could contribute to the susceptibility and manifestation of these diseases, in particular to the control of specific and total IgE responses. The TCRα/δ locus encodes the α and δ chains of the TCR, and the immunoglobulin heavy chain loci is involved in isotype class switching to IgE in B cells. Other candidate genes mapped to this region that play biologically plausible parts in inflammation and allergy include the nuclear factor κ inhibitor, the oncogene Fos, and TGF-β3.

We have found a previously unreported linkage of both D14S75 and D14S63 to atopy ($p = 0.017$ and $p = 0.0042$, respectively) and to IgE ($p = 0.034$ and $p = 0.0029$), which is strengthened by the association of allele 153 of D14S63 to both phenotypes ($p = 0.0042$ and $p = 0.0031$). Furthermore, we have confirmed a previously reported linkage of atopy and antigen specific responses to TCR αδ on 14q11.2 and have shown a new allelic association of D14S67 on 14q32.1 to IgE (0.0023).[60]

We conclude that chromosome 14 may contain three loci predisposing to IgE phenotypes at 14q23, 14q32.1, and 14q11.2.

Tumour necrosis factor

Increased concentrations of TNFα protein have been shown in biopsy samples from the airways of patients with asthma. In addition, airway cells obtained at bronchoalveolar lavage in patients with asthma also show an increased ability to secrete TNFα.[61–64] Studies of peripheral monocytes after specific bronchoprovocation challenge in occupational asthma and alveolar macrophages obtained at bronchoalveolar lavage show increased concentrations of TNFα. Lipopolysaccharide stimulated monocytes from patients with asthma produce threefold greater TNFα than controls.[65–67] Furthermore, increased TNFα has been found in blood and sputum during asthmatic attacks.[68 69]

There are several cellular sources and actions of TNFα. TNFα is increased in nasal polyps, primarily as a result of their high eosinophil content, and is increased sevenfold in mast cells in biopsy samples from patients with asthma.[70 71] TNFα induces IL-6, IL-8, and GM-CSF in bronchial epithelial cells, and in vitro studies have shown that T cells from patients with asthma will adhere more readily to airway smooth muscle cells if the muscle cells have been pretreated with TNFα.[72] This effect is modulated via upregulation of the adhesion molecules ICAM-1 and its ligand LFA-1.[73] In addition, TNFα from alveolar macrophages upregulates ICAM-1 and ELAM-1 on endothelial cells, and ICAM-1 on bronchial epithelial cells.[74–76]

Given the increased concentrations of TNFα in patients with asthma and the suggestion that TNF polymorphisms may be associated with the altered production of TNFα, we examined whether specific alleles of these polymorphic loci may form part of the genetic component of asthma. We studied the LTα NcoI RFLP marker in 556 subjects, of whom 245 had asthma. We identified an association ($p = 0.03$) with wheezing and asthma ($p = 0.08$) and high TNFα secretion haplotype (TNFB*2/B*2) in a subset of individuals.[77] Furthermore, analysis of the −308 TNF2 allele shows a significant association with bronchial hyperreactivity ($p < 0.04$), which is independent of extended HLA haplotypes (Campbell DA, Britton J, Pavord I, *et al*, unpublished data).

Cytokine gene cluster on chromosome 5q

This region of the genome contains several molecules implicated in the pathogenesis of asthma and the control of IgE. The 5q23-31 region includes the genes coding for IL-3, IL-4, IL-5, IL-9, IL-12b, IL-13, glucocorticoid receptor, and the β2 adrenergic receptor. Marsh and coworkers showed in sib pair analysis on Amish families that there is a significant linkage between total IgE concentrations and several markers in this region, namely IL-4R, IRF-1, IL-9, D5S393, and D5S399, of which IL-4R showed the strongest linkage when those subjects with antigen specific responses were excluded ($p = 0.000004$).[78] These results were confirmed using sib pair analysis by Meyers *et al* on Dutch families who showed linkage of total IgE concentrations to IL-9 ($p = 0.07$), D5S393, D5S436, and CSF-1R.[79] Postma *et al* reported linkage of chromosome markers with total IgE concentrations and bronchial hyperresponsiveness.[80] The Dutch–American collaboration group also suggested that two genes account for 78% of the genetic predisposition to high IgE concentrations.[81 82] Interestingly, in these studies total IgE and bronchial reactivity were not co-inherited. In addition, Doull *et al* have found significant allelic association between the 118 allele of the IL-9 microsatellite and total serum IgE.[83] This linkage, however, has not been confirmed by Sandford *et al*,[84] either for bronchial reactivity or for total IgE concentrations, nor by an association and linkage study by our group.

Walley and Cookson presented results on linkage and allelic associations between chromosome 5q markers and atopic asthma phenotypes.[85] They were unable to find evidence of linkage to total serum IgE or to bronchial hyperresponsiveness, but established a significant linkage to eosinophilia (D5S658, $p = 0.00091$). Significant allelic associations were shown for microsatellite alleles D5S1995 and total serum IgE ($p = 0.0035$), IL4RP1, and eosinophil count ($p = 0.0011$), and IL-9 and bronchial hyperresponsiveness ($p = 0.00042$).

At present, there is only evidence for one polymorphism being functional in the 5q cytokine cluster: in the IL-4 promoter.[86] Investigation of this polymorphism for associations with asthma and atopy phenotypes in two populations has shown only a weak association with specific IgE to house dust mite (*Dermatophagoides pteronyssinus*) ($p = 0.013$) in one, but the not the other, population tested.[87]

MHC locus on chromosome 6p

The genetic predisposition to allergen responses causing specific atopic or asthmatic reactions has also focused attention on the possible association with variation in HLA or T cell receptor proteins. These molecules are pivotal in the handling and recognition of antigens. There is much published work showing an association of class II haplotypes and antigen specific responses. Two such associations are with HLA DQA*0101 and specific IgE responses to *Ambrosia artemisiifolia V* (Amb a V), and of house dust mite peptide epitopes with HLA DQB1*0301 and DRB1*1101.[88 89] This restriction has not been universally shown for house dust mite epitopes. An association between HLA-DR1 and Fel d I as well as Alt a I and DR4 has, however, been shown.[90] As with the studies on 5q, there is conflicting data on the linkage of asthma or bronchial responsiveness to HLA. No linkage was detected to chromosome 6p using D6S105 for atopy or bronchial responsiveness in 20 families ascertained through a proband diagnosed as having asthma between 1962 and 1970.[91] With regard to asthma, certain HLA associations have been shown in isocyanate asthma to alleles of HLA DQB1*0503 and

HLA DQB1*0201/0301.[92 93] In the former study a Val/Asp polymorphism at position 57 inferred susceptibility to the disease. In aspirin sensitive asthma, DQB1*0401 protects against disease, whereas Dqw2 may increase the risk.[94] In contrast, HLA DPB1*0401 has been shown to protect subjects from developing allergic asthma in a mulatto population from Columbia, South America.[95]

Chromosome 12q15-q24.1

The presence of the genes INFγ, stem cell factor (also known as mast cell growth factor), insulin like growth factor I, and the β subunit of nuclear factor y makes this region an attractive candidate for asthma and atopy susceptibility loci. Barnes *et al*[96] have reported the linkage of asthma and total serum IgE concentrations on chromosome 12q in two populations: 29 Afro-Caribbean families (ascertained through 29 asthmatic probands) and 11 Amish kindreds. Linkage was detected in the Afro-Caribbean families to asthma at D12S379 ($p = 0.001$) and log[total serum IgE] at D12S360 ($p = 0.001$). The Amish population showed replication at a lower level of significance for log[total serum IgE] at D12S360 ($p = 0.01$). They went on to perform a multipoint analysis of asthma in the Afro-Caribbean families, showing a peak close to D12S379 ($p = 0.003$).

WHOLE GENOME SCREENS FOR ATOPY AND ASTHMA

These loci do not, however, account for all of the genetic predisposition to asthma. Genome wide searches have therefore been carried out by a number of groups, with the first published in two independent populations.[24] The first population showed potential linkage on chromosomes 4, 6, 7, 11, 13, and 16 ($p < 0.001$). Of these linkages on chromosomes 4, 7, and 16 the test statistic met the suggested more stringent criterion of $p < 0.0005$, and, for chromosomes 6 and 11, $p < 0.0001$. Chromosomes 11 and 16 exhibited linkage to IgE concentrations. The marker FCERB located on chromosome 11 and a microsatellite in the β chain of the high affinity receptor for IgE also showed linkage to the allergy defined by skin prick testing. The regions on chromosomes 4 and 7 were linked to bronchial responsiveness and the region on chromosome 6 was linked to an increased eosinophil count. A second screen was performed on a second population using the markers showing $p < 0.001$ for linkage. This showed linkage of asthma to FCERB ($p = 0.003$) and D16S289 ($p = 0.03$) and atopy to D13S153 ($p = 0.003$). Additional statistical testing was performed to determine the presence of parent of origin effects. This indicated significant differences between maternal and paternal alleles. Linkage to maternal meiosis was reported between D4S426 and atopy and total serum IgE, and between D16S289 and atopy ($p < 0.01$) and asthma ($p < 0.001$). FcεRI-β showed maternal linkage to atopy ($p < 0.0001$) and asthma ($p < 0.00001$).

COLLABORATIVE STUDY ON THE GENETICS OF ASTHMA

A separate study has been reported in several ethnic groups using a different definition of the asthma phenotype by the American collaborative study on the genetics of asthma.[97] Linkage was shown to chromosomes 5q23-31, 6p21.3-23, 11p15, 12q14-24.2, 13q21.3, 14q11.2-13, and 19q13 in white sib pairs; chromosomes 5p15 and 17p11.1-q11.2 in African–American sib pairs, and 2q33, 12q14-24.2, and 21q21 in Hispanic sib pairs. The level of significance of linkage used ($p < 0.01$) implies that some will be false positive linkages.

Together these studies show many possible new loci linked to asthma and allergy traits. Only a small number of these loci have been found to have replicated in different studies: chromosomes 5q, 6p, 11q, 12q, and 13q. The characterisation of the genes from all the loci found by the genome surveys remains a daunting task that will take several years of intense effort. The story of the elucidation of the genetic basis of type 1 diabetes mellitus gives a direct indication of the resources required to determine the contribution of individual loci.[98] In fact, the resources required will be greater still as it is unlikely that any one locus will have as significant an effect on asthma as the HLA locus has on diabetes mellitus. The present state of art of asthma genetics research has been reported by Holgate.[99]

Conclusions

Several complementary techniques have now addressed the molecular basis of asthma and allergy. Directed functional studies have defined the cellular basis of underlying asthma and allergy and have implicated IL-4, IL-5, TNF, and GM-CSF as strong molecular candidates.

The study of the basic genetics and cellular mechanisms will produce insights of the aetiology and pathogenesis of asthma. It is hoped that much of this knowledge will prove to have practical clinical value. In particular, questions such as the cause of the apparent increase in the incidence of asthma and why certain environmental factors trigger asthma in some, but not all, subjects exposed need to be answered. Determining the inheritance of a set of polymorphisms in a subject may allow the prediction of those at risk of developing asthma and may allow disease prevention strategies to be more accurately directed. Similarly, the inheritance of a set of polymorphisms may indicate the clinical course, its severity, and its response to treatment. In the future, genetics may result in the development of novel pharmacological and immunological treatment strategies in asthma and allergy with greater specificity.

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