THE IMMUNOGENETICS OF ASTHMA AND ECZEMA: A NEW FOCUS ON THE EPITHELIUM

William Cookson

Abstract | Asthma and eczema (atopic dermatitis) are the most common chronic diseases of childhood. These diseases are characterized by the production of high levels of immunoglobulin E in response to common allergens. Their development depends on both genetic and environmental factors. Over the past few years, several genes and genetic loci that are associated with increased susceptibility to asthma and atopic dermatitis have been described. Many of these genes are expressed in the mucosa and epidermis, indicating that events at epithelial-cell surfaces might be driving disease processes. This review describes the mechanisms of innate epithelial immunity and the role of microbial factors in providing protection from disease development. Understanding events at the epithelial-cell surface might provide new insights for the development of new treatments for inflammatory epithelial disease.

ASTHMA
Intermittent inflammation of the airways of the lung and chronic disease can lead to airway scarring and irreversible limitation of airflow.

ATOPIC DERMATITIS
(eczema). A scaly, itchy rash that typically occurs in the flexures of the elbows and knees, but can also be found anywhere on the body.

Human Genetics, University of Oxford, Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, OX3 7BN, UK.
e-mail: wocc@well.ox.ac.uk
doi:10.1038/nri1500

ASTHMA is an inflammatory disease of the small airways of the lung. Asthma has now reached epidemic proportions, with more than 10% of children being affected in many westernized societies. Infantile eczema (ATOPIC DERMATITIS, AD) is also increasingly common in the developed world, affecting up to 15% of children in some countries. Asthma is present in 60% of children with severe AD, a significant proportion of whom continue with problems into adult life. Both diseases are familial and arise from the interaction between strong genetic and environmental factors.

AD, asthma and hay fever (allergic rhinitis) are often considered to be part of a common syndrome of atopic diseases[1]. Although the term 'ATOPY' has various definitions, it most consistently refers to the presence of immunoglobulin E (IgE)-mediated skin-test responses to common ALLERGENS. Atopic individuals are also typified by the presence of increased levels of total and allergen-specific IgE in the serum.

The scientific study of asthma and its related disorders began around 1900. The success of vaccination against infectious disease meant that the injection of antigens and immune serum was widely used at that time when investigating diseases. These experiments resulted in the discovery of serum sickness (and von Pirquet's coining of the term 'allergy'[2]), WHEAL-AND-FLARE RESPONSES to the injection of antigens in the skin (the basis of modern SKIN-PRICK TESTING for allergen sensitivity), the passive transfer of skin-test sensitivity by serum from atopic individuals, systemic ANAPHYLAXIS, and immunotherapy for hay fever with injections of crude antigen[3]. Although these findings arose from artificial circumstances (the injection of foreign substances), they still distort and sometimes confuse the understanding of asthma and AD today.

The serum factor that transferred skin-test sensitivity to allergens was eventually discovered to be IgE[1]. Allergen-specific IgE binds to mast cells in the skin and lungs through a high-affinity Fc receptor (FcεRI, encoded by *FCERI*). The penetration of allergen into epithelial-cell surfaces causes crosslinking of FcεRI molecules, resulting in mast-cell degranulation and the release of many mediators of inflammation (FIG. 1). IgE-mediated sensitivity has subsequently been considered to be central to the initiation of atopic disease[4] and a large body of research has been directed towards

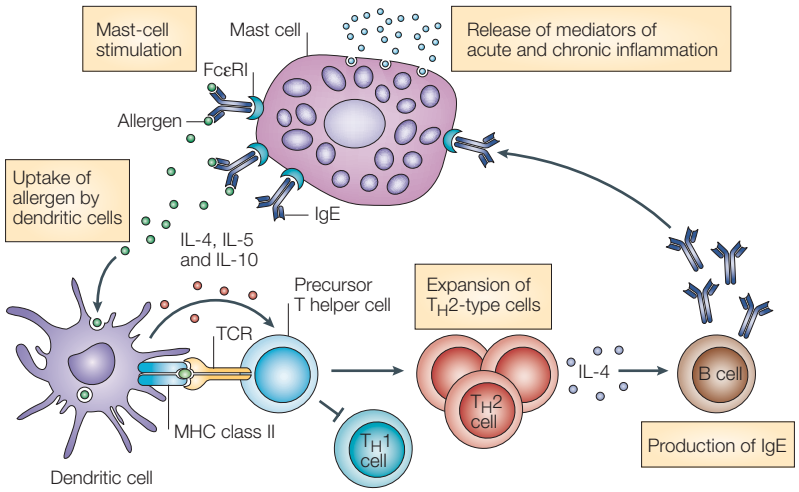

Figure 1 | **Classical mechanisms of atopy in asthma and atopic dermatitis.** Allergens are taken up by dendritic cells and presented to T cells. In the absence of childhood microbial exposure, the balance between T helper 1 (T_H1) and T_H2 cells is altered. T_H2 cells encourage the production of immunoglobulin E (IgE) by B cells. Allergen-specific IgE then binds to the high-affinity receptor for IgE (FcεRI) on mast cells. Allergen exposure induces crosslinking of receptor-bound IgE with subsequent mast-cell degranulation and the release of pro-inflammatory molecules. IL, interleukin; TCR, T-cell receptor.

ATOPY
(meaning 'strange disease'). A term invented to describe the familial syndrome of asthma and hay fever and their association with positive skin-prick tests.

ALLERGENS
Common inhaled proteins that induce allergic responses. Typical allergen sources include house-dust mite, grass pollens and animal danders (droppings from skin and fur).

understanding the humoral and cellular factors that regulate IgE production[5,6].

The effective production of IgE in atopic disease by B cells depends on support by T helper 2 (T_H2) cells, which produce interleukin-4 (IL-4), IL-5, IL-9 and IL-13. In general, T_H1 cells promote cellular rather than humoral immune responses, and they are prominent in other chronic disorders, such as Crohn's disease and PSORIASIS. Cells with the T_H2-cell phenotype have been well studied for their role in the pathogenesis of airway inflammation and asthma, and a well-supported model of asthma pathogenesis denotes that T_H2 cells function as key players by promoting IgE production by B cells[6].

Any model of the immunology of asthma and AD has to take into account the observation that both diseases have increased in prevalence during the past century. It is unlikely that genes that modify susceptibility to these diseases have changed in this short time, so the increase in disease must be attributable to a change in as-yet unknown environmental factors. However, increases in exposure to house-dust mites and other allergens have not been sufficient to explain the increase in asthma prevalence[7], and air pollution has effectively been excluded as a cause of the asthma epidemic[8]. However, asthma prevalence has been linked to increasing hygiene standards and the progressive westernization of lifestyles in many countries, and a protective effect against asthma of microbial exposure in early childhood has been suggested by the 'hygiene hypothesis'[9,10]. This hypothesis argues that early childhood exposure to infections inhibit the tendency to develop allergic disease. As a consequence, children with westernized lifestyles, protected as they are from the infectious burdens of early life that are common in the developing world, suffer an increased risk of developing allergic disease. There is now strong evidence indicating that microbial exposure is important for protection against asthma (BOX 1), although the nature of the microbial protective effect is still unknown.

Several theories have been put forward to explain the association between asthma and hygiene. The theory of immune deviation suggests that atopic asthma is initiated soon after birth, when the naive immune system is first confronted with potentially allergenic airborne antigens[11]. It is suggested that the initial phase of allergen exposure results in compartmentalization of immunological memory into either T_H1- or T_H2-cell phenotypes in non-atopic and atopic individuals, respectively. Microbial exposure in infancy encourages a milieu in which initial allergen exposures produce benign T_H1-cell responses. In the absence of such exposure, T_H2-cell responses predominate, and can be followed by chronic T_H2-cell-driven inflammation in the airways[11]. This raises the possibility that manipulation of the immune system in early life could result in persistent T_H1- or T_H2-type responses. If this is the case, vaccination to induce T_H1-cell responses might be effective against asthma and other allergic disorders[12]. As an alternative to the immune deviation theory, it has been proposed that lack of 'normal' microbial exposure leads to reduced activity of regulatory T cells rather than T_H2-cell deviation[13].

The current view that high IgE levels driven by T_H2-cell responses account for the pathogenesis of atopic disease has several inconsistencies. First, 10% of cases of childhood asthma or of AD have normal levels of total IgE and do not have specific IgE responses to common allergens[14]. These patients are, however, clinically indistinguishable from individuals with diseases characterized by elevated IgE levels[1]. Furthermore, immunotherapeutic strategies that block allergen-specific IgE responses[15–17] or that remove total serum IgE[18] are of incomplete efficacy for the treatment of atopic asthma and AD, indicating that IgE-independent mechanisms probably contribute to the presence of both diseases.

The second problem relates to the initiation of disease by exposure to allergens. It is assumed that allergens are important primarily because they are allergenic, and that they produce allergic responses because they are respirable (that is, able to penetrate into the respiratory tract) and soluble. However, as discussed later, the

Box 1 | **The hygiene hypothesis**

Atopic asthma has increased in frequency over the past 100 years, and a high prevalence of asthma and atopy is found in westernized and urbanized societies. Protective effects against asthma and atopy include

- **Living with pets**[127,128]
- **Living with several brothers and sisters**[9,127,129]
- **Drinking unpasteurized milk**[130,131]
- **Living on a farm**[130]
- **Living in close proximity to farm animals**[130]

These findings indicate that

- **Asthma and atopy have a strong environmental component**
- **Abundant microorganisms in the childhood environment seem to be protective**
- **Manipulation of the environment might prevent asthma**

presence of foreign proteins at mucosal surfaces is, on its own, insufficient to produce an immune response, and other factors have to be invoked to explain why allergens are associated with disease.

Given that the basic causes of asthma and AD are still unclear, it is proving increasingly helpful to understand their genetic basis. Genetic experimentation offers a structured approach to identifying unknown genes and pathways, and the genes so far identified in asthma and AD are beginning to close some of the gaps in our understanding of these diseases. This review describes what is known about the genetics of asthma and AD, and then suggests potential mechanisms to explain the interaction between genes and the environment in disease pathogenesis.

Immunogenetics

In general, the study of genetics is the study of polymorphism. However, not all genes are polymorphic, such as those that are crucial to normal development, and not all disease pathways will be discovered using a genetic approach. Nevertheless, survival in a hostile environment characterized by constantly evolving threats from microorganisms depends on spending considerable energy on evolving and maintaining immunity. Consequently, many genes and proteins of the immune system are polymorphic.

Although disease can result from mutations in single genes, most of the common diseases with familial clustering are due to complicated interactions between an unknown number of genes and environmental factors. Genetic research into these 'complex diseases' or 'complex traits' has begun to make a substantial contribution to the understanding of mechanisms for illnesses such as Alzheimer's disease, diabetes, asthma and inflammatory bowel disease (IBD). Functional polymorphisms might be expected in many genes that influence immunity, and many of these might show associations with disease if they are tested carefully enough. However, it seems likely that complex diseases are most influenced by a few polymorphisms that have a large effect[19], and it is these polymorphisms that are of particular interest in the unravelling of disease pathogenesis.

Genome screens of asthma and AD

As childhood asthma runs strongly in families, and studies of twins with asthma or AD show a heritability of approximately 60% (REFS 20–22), many GENOME SCREENS have been carried out to search for genetic effects on these illnesses. Genome screens involve the study of families containing individuals with the disease of interest. In these familial screens, evenly spaced genetic polymorphisms (markers) that cover all the chromosomes are typed in family members, and a search is made for genetic regions that are co-inherited with disease (a phenomenon known as GENETIC LINKAGE). Linked regions (loci) are then mapped in detail to identify the underlying 'disease gene' or genes.

At least 11 whole-genome screens have been reported for asthma and its associated phenotypes (reviewed in REFS 23,24). These have identified ten regions of linkage that were reproducible between screens and four regions that were statistically significant but not replicated by other groups[23]. Those regions that were consistently identified are likely to contain the genes with the strongest effect on disease (FIG. 2).

Genome screens have also been carried out for many other immune diseases that have a genetic basis and have identified regions of linkage that are shared[25] (FIG. 2). Asthma, for example, consistently shows linkage to the MHC loci on the short arm of chromosome 6 (REF. 26) — as with many other diseases — and linkage loci for asthma also overlap with loci for other inflammatory and autoimmune diseases, such as ankylosing spondylitis (on chromosomes 1p31–36, 7p13 and 16q23); type 1 diabetes (on chromosomes 1p32–34, 11q13 and 16q22–24); and multiple sclerosis and rheumatoid arthritis (on chromosome 17q22–24) (REF. 26). These findings indicate that the susceptibility to different diseases arising from these loci might be influenced by individual genes in various forms (alleles). Alternatively, as in the case of the MHC loci, disease susceptibility might be modified by physical clusters of genes that have many effects on immune responses. In either event, the mapping of these shared loci is of particular interest, as they indicate that pathways might be common between diseases and, therefore, might lead to the elucidation of as-yet unknown immune processes.

Three genome screens for AD have been carried out and have identified significant linkage to AD in four regions (reviewed in REF. 27). However, these regions do not overlap with known regions of linkage to asthma, indicating that susceptibility to asthma and AD are mediated through different genes rather than through a common atopic background. The four AD loci are, however, closely coincident with regions that are known to contain genes that are associated with psoriasis susceptibility[27], and one of the shared loci, on chromosome 20p, also shows strong linkage to leprosy susceptibility in families from a region of endemic leprosy in South India[28]. This indicates that particular genes or families of genes have general effects on immune reactions in the skin.

Single-gene disorders: Netherton's disease

The POSITIONAL CLONING of novel genes from regions that are linked to complex diseases is a long and difficult task. By contrast, the identification of Mendelian (single-gene) disorders is much more straightforward, and can sometimes give insight into complex diseases.

An example of a single-gene disorder is Netherton's disease, a rare recessive disorder that is characterized by a generalized congenital erythroderma. Importantly, children with this syndrome consistently develop symptoms of atopic disease (hay fever, food allergy, urticaria and asthma) and high levels of IgE in the serum, and might therefore shed light on common disease pathways[29,30]. In 2000, mutations in the gene encoding a serine protease inhibitor known as SPINK5 (also known as LEKTI) were shown to cause disease in patients with Netherton's disease[31,32]. Subsequent work has shown

WHEAL-AND-FLARE RESPONSE
The acute response of the skin to an allergen in a skin-prick test. The wheal is a swelling of the epidermis around the site of the prick (usually several millimetres in diameter). The flare is a reddening of the skin over a wider area that is induced by neuronal mechanisms.

SKIN-PRICK TEST
The introduction of minute amounts of allergen into the epidermis by a prick or scratch induces mast-cell degranulation if allergen-specific IgE is present. A wheal and flare is visible if degranulation takes place, and its size is used as a measure of an individual's allergen sensitivity.

ANAPHYLAXIS
The generalized release of histamine and other inflammatory mediators following systemic induction of mast-cell degranulation by allergen. Anaphylaxis can cause bronchospasm, cardiovascular collapse and death.

PSORIASIS
The most common skin disease of adults, typically affecting the extensor surfaces of elbows and knees but can also be generalized. It is not associated with atopy.

GENOME SCREEN
The systematic localization of chromosomal regions that are co-inherited with disease. Typically, a panel of markers covering all the chromosomes are genotyped in multiple families containing individuals with the disease.

GENETIC LINKAGE
The identification of a chromosomal region that is co-inherited with disease in families.

POSITIONAL CLONING
The process of systematically identifying disease genes from the study of families. Positional cloning begins with genetic linkage regions, which can cover 20–30 million base pairs of DNA and contain 300 genes. The region is refined to 5–10 genes by genetic 'fine mapping', and the remaining genes are studied individually to determine whether they contribute to disease.

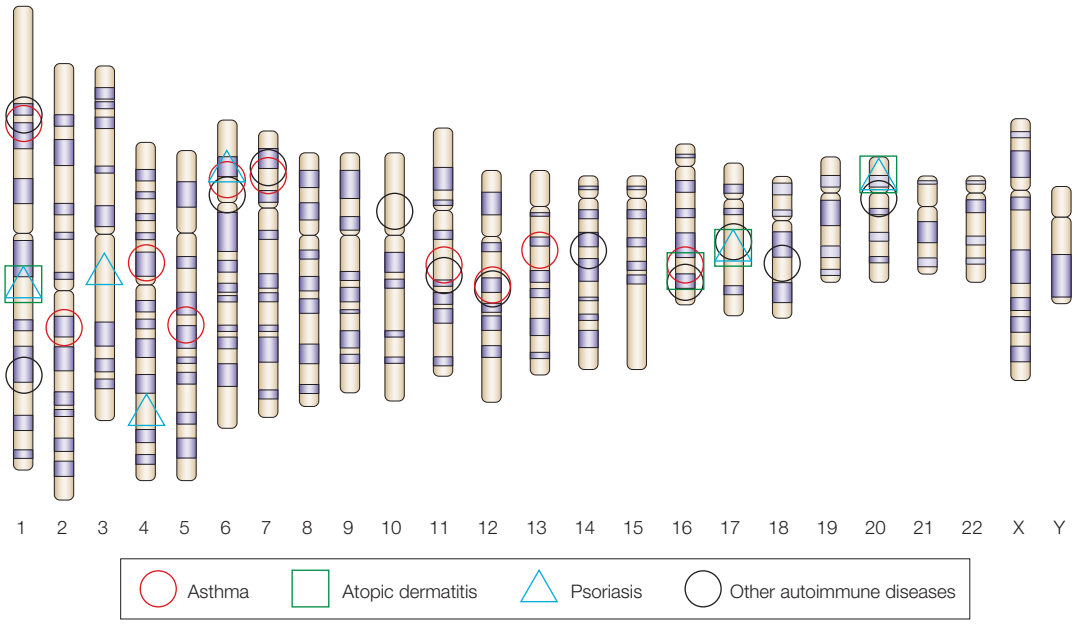

Figure 2 | **Susceptibility loci identified by genome screens for asthma, atopic dermatitis and other immune disorders.** Only loci with significant linkages are indicated. Clustering of disease-susceptibility genes is found for the MHC on chromosome 6p21, but also in several other genomic regions. Note that several loci for atopic dermatitis and psoriasis overlap.

that a common polymorphism in *SPINK5* (particularly Glu420Lys) modifies the risk of developing AD, asthma and elevated levels of serum IgE[33–36], indicating that SPINK5 might be involved in an unexpected pathway in the development of atopic disease.

The SPINK5 protein contains 13 active protease inhibitory domains, which are joined together by linking domains. The sequence of each of the SPINK5 protease inhibitory domains is slightly different[32], indicating a polyvalent action against multiple substrates. SPINK5 is expressed in the skin in the outer epidermis, the sebaceous glands and around the shafts of hair follicles[37], indicating that it might be important for the inhibition of environmental proteases, such as those that arise from bacteria or allergens that penetrate the skin. This is consistent with the observation that a third of patients with AD suffer from frequent, severe bacterial infections in the skin, and the lesions of over 90% of AD patients are colonized with *Staphylococcus aureus*[38,39]. Moreover, nearly all strains of *S. aureus* from skin lesions of AD have high levels of proteolytic activity[40], which is in contrast to the low levels that are typical of control strains isolated from healthy carriers[40], indicating that SPINK5 might be important in suppressing this proteolytic activity.

Many children with AD have high titres of IgE specific for allergens from the ubiquitous house-dust mite *Dermatophagoides pteronyssinus*, so named because it feeds on human skin that is shed from the outermost cornified layer. The main house-dust-mite allergens (*Der p* I and *Der p* II) are present in its faecal pellets and are proteases that have profound effects on epithelial cells, including disruption of intercellular adhesion, increased paracellular permeability and initiation of cell death[41]. If these external sources of proteases are important in disease pathogenesis, then inhibition of protease activity might be a new approach in the therapy of AD. Indeed, encouraging results have been obtained from a small study in which α1-proteinase inhibitor was effective in the treatment of AD[42], although more comprehensive studies are now required.

CORNIFIED ENVELOPE
The cell walls of fully differentiated keratinocytes, which form the stratum corneum.

The epidermal differentiation complex

Genetic linkage studies of AD and of psoriasis have highlighted the importance of chromosome 1q21 (REFS 43,44). This chromosome region contains a collection of genes known as the epidermal differentiation complex (EDC)[45]. Many of these genes have shown increased expression in the skin of patients with AD[46] and psoriasis[46–48]. Although polymorphisms in individual genes of the EDC have not yet been associated with disease, it has been established that disease susceptibility alleles are contained within the cluster[44].

Several gene families are present within the EDC: these encode small proline-rich region proteins (SPRRs), S100A calcium-binding proteins and late envelope proteins (LEPs)[45,49]. The SPRR and LEP families of genes encode precursor proteins of the CORNIFIED ENVELOPE and are involved in keratinocyte terminal differentiation[49,50]. Expression of the EDC proteins occurs late during the maturation of epidermal cells[51], and like SPINK5, proteins of the EDC are mainly localized just beneath the cornified envelope[52].

Global gene-expression studies have been used to investigate the skin lesions of active psoriasis[47,48]. In these studies, 30 of the genes in and around the EDC were differentially expressed when normal skin and skin lesions were compared, with several S100A and SPRR

Table 1 | **Products encoded in the EDC, and their functions**

Protein	Function	References
S100A2	Chemotactic agent for eosinophils	132
S100A7 (psoriasin)	Chemotactic agent for for CD4+ T cells and neutrophils. Antimicrobial activity	113,133
S100A8–S100A8 homodimer	Chemotactic agent for leukocytes	134–136
S100A8–S100A9 heterodimer	Cytostatic activities. Antimicrobial activities. Inhibitor of macrophage activation. Inhibitor of immunoglobulin synthesis by B cells	114,115, 137–140
S100A12	Pro-inflammatory activity towards endothelial cells and inflammatory cells. Filaricidal and filariastatic activity	117,141

EDC, epidermal differentiation complex.

family members upregulated in psoriatic skin[47,48]. The expression pattern of particular genes of the EDC in AD has not yet been described in detail, but is has been observed that *SPRR2C* is expressed at a level that is ten-times higher in psoriasis than in AD, and that *S100A12* gene expression is three-times higher[46].

The functions of some of the EDC gene products indicate that the skin is not functioning as a passive barrier. Accordingly, the S100A calcium-binding proteins are often secreted in response to inflammation and have a wide range of immunological actions[53] (TABLE 1). Given that the skin is the primary interface of the body with the external environment and that it can initiate a range of responses to a variety of insults, it is now of interest to investigate the role of these proteins in inflammatory diseases.

Genes for asthma and other epithelial diseases

Asthma, psoriasis, Crohn's disease and other diseases that affect epithelial surfaces all have a strong genetic component. The positional cloning of susceptibility genes for asthma has been remarkably successful recently, with the identification of four novel genes: *DPP10* (dipeptidyl peptidase 10; chromosome 2q14)[54], *GPRA* (G-protein-coupled receptor for asthma susceptibility; chromosome 7p14)[55], *PHF11* (plant homeodomain finger protein 11; chromosome 13q12)[56] and *ADAM33* (a disintegrin and metalloproteinase 33; chromosome 20p)[57].

The functions and activities of these genes are so far poorly understood, but they do not fit into classical pathways of asthma pathogenesis. *ADAM33* is expressed in bronchial smooth muscle, and is thought to alter the hypertrophic response of bronchial smooth muscle to inflammation (a component of airway remodelling)[57]. *PHF11* encodes a nuclear receptor that is part of a complex containing a histone methyl transferase (SETDB1), a regulator of HDAC (RCBTB1) and a nuclear transport molecule (karyopherin α3). Their function in asthma is unknown. *DPP10* encodes a prolyl dipeptidase, which can remove the terminal two peptides from certain pro-inflammatory chemokines. It is uncertain whether this would activate or deactivate them, but if the substrate for DPP10 is what has been predicted, and if chemokines are activated, then DPP10 might be the target for a new asthma therapy[54]. *GPRA* encodes an orphan G-protein-coupled receptor with isoforms that show distinct patterns of expression in bronchial epithelial cells and smooth muscle cells in asthmatic versus healthy individuals[55]. In general, G-protein-coupled receptors are good targets for pharmaceutical therapy, but more needs to be known about GPRA before it can become a focus for treating or preventing asthma.

Although their functions are still largely unknown, it is of interest that the expression of both DPP10 and GPRA is concentrated in the terminally differentiating bronchial epithelium — the epidermal layer that corresponds to the site of highest expression of SPINK5 and proteins encoded in the EDC (FIG. 3). This indicates that all of these proteins might play a part in the maintenance of the epithelial barrier or that they might be involved in the first lines of response when this barrier is breached.

Studies of CANDIDATE GENES have also identified genes that might be involved in asthma susceptibility, many of which might exert their effects in the mucosa. For example, *IL-13* polymorphism influences mucus production as well as serum IgE levels[58] through a receptor encoded by the polymorphic *IL-4R*[59]. *FCERIB* variants modify the activity of FcεRI on mast cells, possibly by modulating the level of expression of the receptor on the cell surface[60,61]. A receptor expressed by T cells for the key mast-cell signalling factor prostanoid DP has also been reported to be associated with asthma[62]. These findings indicate that the role of mast cells in epithelial inflammation might also be a potential target in asthma therapy.

Other asthma susceptibility genes include the pattern-recognition receptors (PRRs) of the innate immune system, which are expressed by dendritic cells and other cells, and recognize specific microbial components and activate innate immune responses. Polymorphism in *CD14* (REF. 63), *TLR2* (Toll-like receptor 2) (REF. 64), *NOD2* (nucleotide-binding oligomerization domain 2, also known as *CARD15*) (REF. 65) and *TIM1* (T-cell immunoglobulin domain and mucin domain 1, also known as *HAVCR1*) (REF. 66) have all been shown to influence asthma susceptibility, indicating that these genes might be important in providing the link between microbial exposure and reduced susceptibility to asthma. Whereas *CD14* polymorphisms have been associated with total serum IgE levels[63], *TLR4* does not seem to be associated with asthma[67,68], and although *TLR2* polymorphisms have been associated with asthma in children raised on farms[64], they do not seem to be associated with asthma in the general population[68]. *TLR10*, which responds to an unknown ligand, has recently been associated with asthma[69]. However, none of these studies has tested for IgE responses to particular allergens, so systematic studies of PRR activation in asthma and AD are now needed.

Other recognized effects are from tumour-necrosis factor (TNF)[70], which encodes a potent pro-inflammatory cytokine that is released by many cells, including airway epithelial cells and transforming growth factor-β (encoded by *TGFB*)[71], which is an important local regulator of epithelial inflammation.

CANDIDATE GENES
Known genes that are investigated for a role in disease by the comparison of polymorphisms in patients and controls.

Several other recent observations have indicated the importance of proteins that are expressed by epithelial cells in conferring susceptibility to (or protection against) disease. Terminally differentiating keratinocytes express psoriasis-susceptibility genes *SLC9A3R1* (also known as EBP50), recently cloned from the psoriasis-susceptibility locus 2 (*PSORS2*) on chromosome 17q25, and *SLC12A8* from chromosome 3q21 (REF. 72) (FIG. 3). *PSORS1*, on chromosome 6p, is another psoriasis-susceptibility locus. This region is complex because of the presence of multiple genes in tight linkage disequilibrium that are possibly co-regulated, and genetic studies have implicated both *HLA-Cw*0602* and the neighbouring gene encoding corneodesmosin (CDSN) in mediating the susceptibility to psoriasis[73–76]. CDSN is also expressed by terminally differentiating keratinocytes and is a key linking component in the STRATUM CORNEUM, making it vital for the maintenance of an effective physical barrier[76].

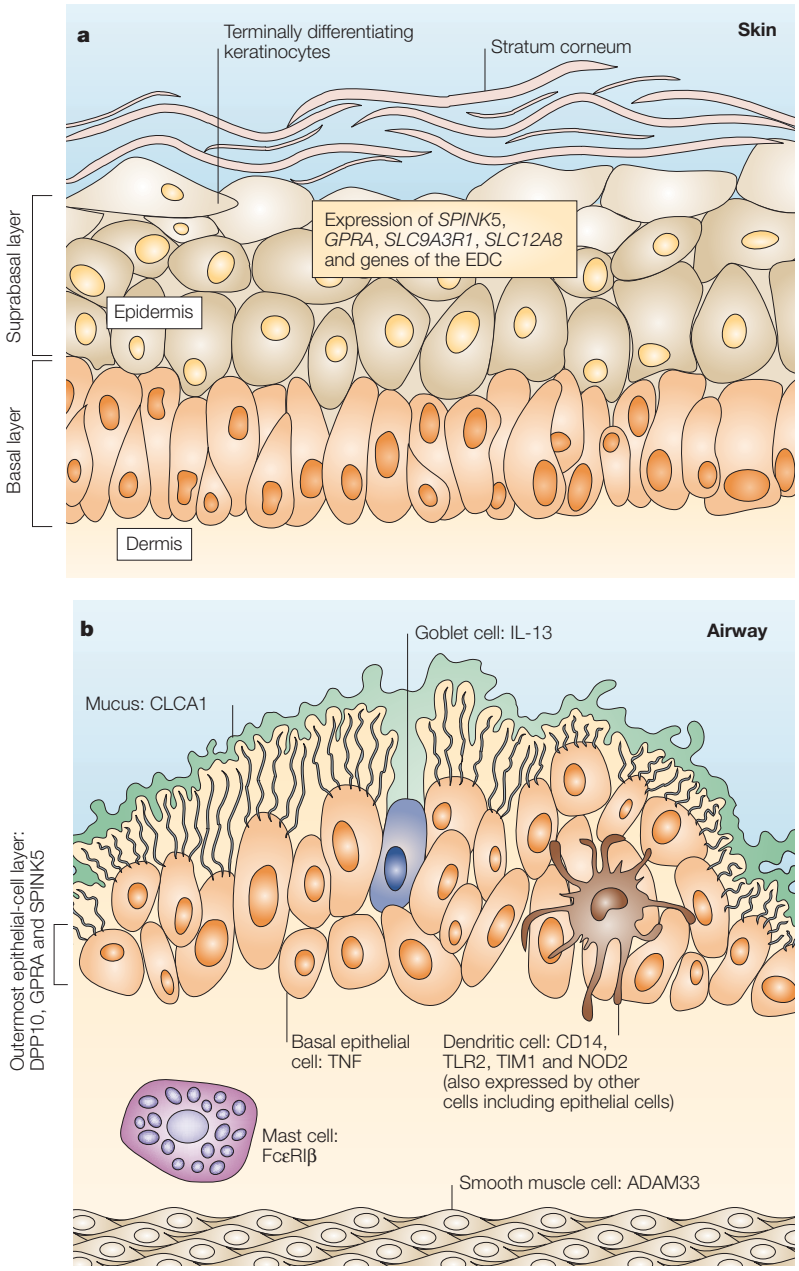

Figure 3 | **The distribution of genes causing susceptibility to asthma and atopic dermatitis. a** | In the skin, proteins encoded by *SLC9A3R1*, *SLC12A8*, *SPINK5*, *GPRA* and the genes of the epidermal differentiation complex (EDC) are found in terminally differentiating keratinocytes. **b** | In the airways, proteins encoded by *DPP10*, *GPRA* and *SPINK5* are found in the terminally differentiated airway epithelium. The function of all of these positionally cloned genes is uncertain. Pattern recognition receptors for microbial products such as CD14, TLR2, TIM1 and NOD2 are expressed by various cells, including dendritic cells and epithelial cells. ADAM33 is expressed in bronchial smooth muscles, and is believed to modify bronchial responsiveness to allergens. The recently identified cation transporter CLCA1 is detected at high levels in the mucus. TNF, tumour-necrosis factor.

Susceptibility to IBD is conferred by *NOD2* on chromosome 16p[77,78], *DLG5* on chromosome 10q23 (REF. 79) and organic cation transporter (OCTN) genes (*SLC22A4* and *SLC22A5*) on chromosome 5q31 (REF. 80). *NOD2* mutants interfere with the function of Paneth cells, which are most numerous in the terminal ileum and are critically important in enteric antibacterial defences[81,82]. The highest levels of expression of *DLG5* and OCTN genes are in terminally differentiating epithelial cells. *DLG5* encodes a scaffolding protein involved in the maintenance of epithelial integrity[79] (FIG. 3).

OCTN genes (*SLC22A4* and *SLC22A5*) are predicted to be cation transporters on the basis of their sequence homologies, but they do not have known substrates or functions. Other equally mysterious cation transporters that are involved in the genetic predisposition to epithelial disease include *SLC12A8* and psoriasis (described above), and *CLCA1*, which has recently been implicated in asthma[83] and CHRONIC OBSTRUCTIVE PULMONARY DISEASE[84], and is found at high levels in the mucus.

The list of susceptibility genes so far discovered for each of these diseases contains many surprises and many unanswered questions. However, the expression of many of these genes in the skin or mucosa indicates that their function lies in these tissues. Although previous understanding of asthma, AD, psoriasis and IBD has centred on mechanisms in the adaptive immune system, often with an emphasis on the T_H1–T_H2 paradigm, the results from genetic studies indicate that further understanding of innate mechanisms of epithelial defence is essential to the treatment and prevention of these disorders.

Mechanisms of epithelial immunity

In evolutionary terms, epithelial surfaces have had to cope with infections and other insults long before the emergence of the adaptive immune system, and most life on earth still exists without the help of an adaptive immune system. Immunity must first have evolved in the primitive epithelium, and the subsequent evolution of specialized immune effector cells of the innate and adaptive immune system has built on mechanisms initially developed in epithelial cells.

It should therefore not be surprising that gene-expression studies indicate that epithelial cells are immunologically active. Keratinocytes in the skin are the most-studied epithelial cell and are known to produce a wide range of cytokines[85]. Although this activity has been assumed to be secondary to signalling from classical

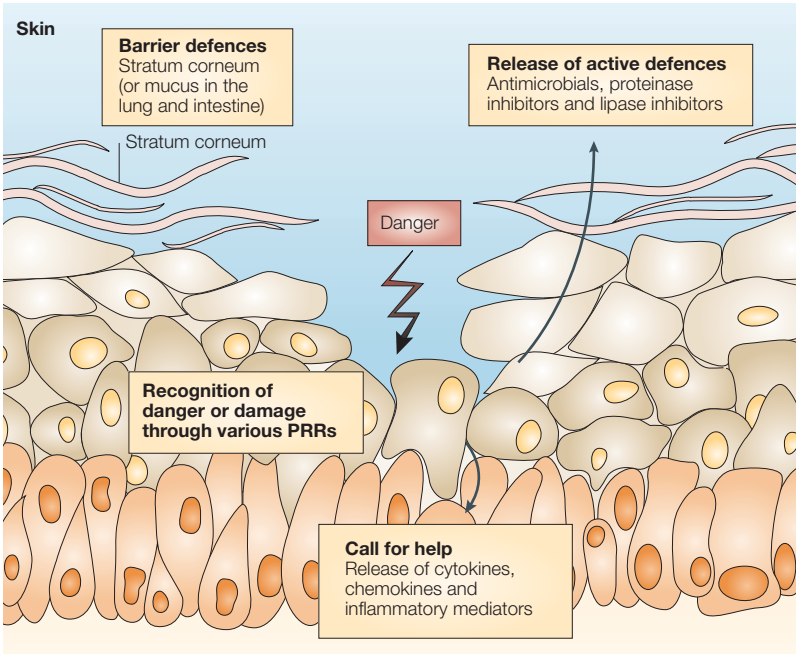

Figure 4 | **Defence mechanisms in epithelial cells.** Epithelial cells resist damage owing to the stratum corneum of the skin or the mucus in the airway or intestine. Immune responses to external antigens are only induced in the presence of danger and damage. Danger is recognized through pattern-recognition receptors (PRRs), with the resultant release of active defences, such as antimicrobials or antiproteinases, and signalling molecules to recruit help from specialized immune cells. Different receptors recognize different microbial products or other factors, and can modify the immune-signalling millieu appropriately. In this model, T helper 1 (T_H1)- or T_H2-cell responses are driven by the nature and site of the initial injury.

immune cells[86], keratinocytes express functional receptors such as CD14 and TLR4 — which recognize lipopolysaccharides — and can induce pro-inflammatory responses without pre-induction by other cells[87]. Airway epithelial cells also express PRRs[88], and cultures of pneumocytes show marked alteration of expression of cytokines, DNA-binding proteins and nuclear factor-κB (NF-κB)-regulated genes after exposure to respiratory pathogens[89,90].

The mechanisms through which the epithelium interacts with the environment, including barrier defences, the recognition of danger and the call for help from specialized inflammatory cells are discussed below (FIG. 4).

Barrier defences. The simplest form of defence consists of putting up a mechanical barrier to bacterial entry and bacterial proteolysis. The ubiquitous presence of bacteria on the surfaces of the body means that an effective barrier needs to be augmented by protease inhibition and by bacteriostatic and antibiotic molecules. Numerous components that make up this barrier have now been defined, many of which are encoded in the EDC on chromosome 1q21.

The location of SPINK5 expression and its polyvalent activity indicate that it might be an important component of the epithelial barrier. Other protease inhibitors with similar roles are encoded by a cluster of genes on chromosome 20q12 (REF. 91), close to a region that has been linked to AD and asthma[43]. These proteins, which have homology to whey acidic protein (WAP), are produced at epithelial-cell surfaces and include elafin and secretory leukocyte protease inhibitor (SLPI)[91]. Elafin is a component of the cornified envelope, and has antimicrobial activity against Gram-negative and Gram-positive bacteria[92]. SLPI is found in airway surface fluid, where it might have a prominent role in mucosal defences against microbial attack[93], as it has been shown to be a potent antimicrobial agent with antiretroviral, bactericidal and antifungal activity[94]. However, the functions of other members of the WAP cluster have not yet been studied.

The importance of airway surface fluid and airway mucus is often neglected in the current understanding of allergic processes. In addition to SLPI, the airway surface fluid contains other small antimicrobial peptides such as β-defensins and cathelicidins[95–97], which are secreted in response to damage and danger signals, and significant antimicrobial activity is also conferred by lysozyme and lactoferrin[93,98]. Whereas the airway mucosa produces high levels of immunoglobulins, particularly IgA, as shown by microarray analyses of gene expression in the nasal mucosa[99], deficiency or low levels of IgA are associated with an increased prevalence of atopic disease[100,101]. Failure of nonspecific components of the epithelial barrier, such as IgA or SPINK5, can therefore give rise to increases in serum IgE and the manifestations of atopy. This suggests a hypothesis in which IgE responses to allergens are either the result of an inability to prevent allergens from breaching epithelial surfaces, or are the result of secondary penetration of allergens into epithelial-cell surfaces that have already been damaged by other factors.

The concept of a general barrier failure in atopic disease helps to explain why more than 95% of the serum IgE in most individuals is specific for unknown antigens, why many individuals with asthma and AD do not have atopy, and why specific immunotherapy and treatment with IgE-specific antibodies do not abolish asthma or AD in patients with atopy.

Danger recognition. Before an organism can respond to infection and damage, it first has to recognize that danger and damage are present. Microbial infection is recognized by PRRs that respond to a wide variety of pathogen-associated molecular patterns (PAMPs). In addition to the recognition of microbial antigens, the initiation of an immune response also requires 'danger' signals produced by injured tissues[102,103]. Many PRRs, including pulmonary collectins, surfactant-associated proteins A and D, C-reactive protein (CRP) and soluble CD14 exert their effects by binding microbial molecules and facilitating their neutralization or degradation by specialized cells[104]. These molecules do not contain signalling motifs. However, other PRRs do produce intracellular signals when they bind to particular microbial ligands. The TLRs are the most-studied of these, and are now known to induce specific reactions to a wide variety of bacterial and fungal components[105].

STRATUM CORNEUM
The mechanically and chemically resistant outermost layer of the skin, which is made up of a complex mixture of lipids and proteins.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE
A disease of irreversible airway constriction. It usually results from a combination of cigarette smoking and asthma.

Intracellular PAMPs, particularly peptidoglycans, are recognized by the NOD/CARD family of proteins[106]. The NOD/CARD proteins are themselves part of a wider family of NBS-LRR (nucleotide binding site and leucine-rich repeat) proteins that bind microbial products through their LRRs and bear structural similarities to PRRs in plants[107]. This family alone contains over 40 members, and the list of PRRs will probably continue to grow.

Additional endogenous danger signals that arise from cell damage (heat-shock proteins and cardiolipin) (reviewed in REF. 108), also activate PRRs and amplify the immune response.

The initial immune response to allergens now needs to be examined in the context of these innate mechanisms that have evolved for responding to foreign antigens. The requirement of tissue injury to trigger an immune response means that allergens cannot be considered soluble proteins that accidentally induce immune responses, as they appear in the wrong place. It is more likely that they excite an immune response because they damage epithelial integrity. The toxic effects of *Der p* I and *Der p* II from house-dust mites are well documented[41], and the observations that *Fel d* I, the main cat allergen, degrades denatured collagens and cleaves fibronectin[109] and that the main grass allergen *Phl p* V is an RNAase[110], favour the hypothesis of epithelial damage by allergens. If allergen damage to the airways or skin initiates disease through innate pathways, then treatment strategies to circumvent specific adaptive antigen-driven immune reactions are unlikely to be successful.

The call for help. The recognition of danger is followed by the induction of active defences against microorganisms and the recruitment of help from specialized cells of the innate and adaptive immune systems. Many molecules secreted by epithelial cells in response to danger have the ability to regulate immune reactions and recruit cells of the innate and adaptive immune systems. These include S100 proteins from the EDC, chemokines, the human cathelicidin cationic antimicrobial peptide LL-37 (REF. 111), and α- and β-defensins, which possess structural and signalling similarities to chemokines[112].

Recent studies have indicated that the novel asthma gene *DPP10,* owing to its peptidase activity, might serve as a checkpoint in the activation or deactivation of chemokines that have an Xaa-Xaa-Pro-Xaa-Ser motif at their amino (N)-terminal[54]. Chemokines with this motif include SDF1α, IP10, eotaxin and RANTES[54]. These putative substrates are active in asthmatic airway inflammation, tentatively indicating that DPP10 might be a target for the therapy of asthmatic airway inflammation.

It is of interest that many of these early signalling molecules also have antimicrobial activities as well as the ability to regulate immune reactions. Molecules with this dual function include several S100A proteins[113–117] and about two-thirds of known chemokines[118]. This duality of function might be an indication of the evolution of immune signalling from molecules that were originally secreted solely for defence against microbial insults.

Early innate modulation of T_H1 or T_H2 cells

The nature of the immune response is first influenced by the specific signals that are involved in the early recruitment of immune components to the site of inflammation. As different PRRs can signal through different pathways, different pathogens or antigens can induce different immune responses[104,119]. Second, the nature of the local immune response might also be strongly influenced by tissue-specific factors, and it has been suggested that epithelial cells, in general, tend to initiate T_H2- rather than T_H1-type responses[103]. In addition, there is evidence that dendritic cells from airways encourage T_H2-cell development by default[120], and that the induction of T_H2- or T_H1-type responses by dendritic cells depends on the stimulus with which they are activated[121].

The perception that specific early signals induced by different infections (or damage by different proteins or other entities) might modify the nature of the subsequent immune response has implications for the T_H1–T_H2 paradigm of atopic disease. One important issue is the timing of establishment of the T_H2-cell bias: on the one hand, T_H1- or T_H2-cell responses to allergens might be fixed at the time of first exposure in early childhood, and the bias might be subsequently manipulated by bacterial or other adjuvants. On the other hand, T_H1- or T_H2-cell responses might develop as a consequence of activation of particular PRRs by particular PAMPs that are present in allergens.

The therapeutic options are different for the two possibilities. If T_H2-cell responses to allergens are the default mechanism, then the ability to modulate the T_H2–T_H1 balance by therapeutic methods is diminished. Alternative therapeutic possibilities might stem from examination of the specific events that are induced by allergen contact with epithelial-cell surfaces. These might include boosting of the epithelial barrier with protease inhibitors, blocking of particular PRR ligand-binding sites and their downstream signals, or interference with the early signals of inflammation, such as those potentially identified by positional cloning studies.

Microbial protection against atopy

Although the current emphasis in understanding asthma and AD is now moving from involvement of distant adaptive immune responses to local responses at epithelial-cell surfaces, it is probable that a full understanding of these diseases will also depend on studies that include the commensal bacteria.

Current understanding of the hygiene hypothesis rests on the suggestion that microbial stimulation during early life is essential for the normal development of the immune system and to achieve the 'correct' cytokine balance[122]. However, the evidence described earlier indicates that damage to the epithelium is probably the initiating event in atopic disease, and the T_H1- or T_H2-cell bias of subsequent inflammation might be secondary to the nature of the damage.

Alternative mechanisms for bacterial products to modify the risk of atopic disease include the enhancement of an effective airway barrier by the induction of mucus production through IL-13 stimulation[58], or the induction of sufficient polyclonal IgA or IgE to provide nonspecific protection against allergens. Additionally, a protective role by microorganisms might follow the acquisition of distinct commensal or symbiotic organisms. Once an individual's commensal microflora is established in the first year of life it remains relatively stable[123,124]. Substantial differences have been observed in the intestinal microflora between neighbouring countries with a different prevalence of atopic disease[125], and between atopic and non-atopic children living in each of these countries[126]. As commensal and symbiotic organisms actively manipulate host immunity and the activity of other bacteria[124], it should be considered that interactions among commensal bacteria, pathogens and the host might contribute to the increase in prevalence of asthma and AD.

Concluding remarks

Although our knowledge of host-susceptibility factors to asthma and AD is still incomplete, involvement of proteins in the outermost layers of the skin and mucosa is a consistent theme that is emerging from genetic studies of these and other epithelial-cell diseases. In addition to specific questions raised by the discovery of individual genes that contribute to asthma susceptibility, such as *DPP10* and *GPRA*, a focus on the epithelium encourages investigation of the mechanisms by which allergens damage the epithelium and the danger signals and PRRs that they activate. The profound effects of *SPINK5* mutations on the development of Netherton's disease indicate that high IgE levels and symptoms of AD or asthma might result from failure of the epithelial barrier, and that damage from non-allergens such as *S. aureus* in AD might be important in driving disease in some circumstances.

The initiation and maintenance of inflammation at epithelial surfaces is induced by local mechanisms that have marked effects on the outcome of the immune response. Genetic studies indicate the presence of many previously unknown or ignored molecules, such as the S100 proteins from the EDC, which might be novel targets for the control and suppression of epithelial inflammation.

A central mystery of asthma revolves around the protective effect of microbial exposures in childhood. This effect has mainly been investigated in the context of T_H1- or T_H2-biased responses, but it might also be explicable by mechanisms confined to epithelial-cell surfaces. Some genetic factors, such as *CD14* and *TLR2*, have been identified that might interact with this microbial environment. The full characterization of genes interacting with microorganisms in asthma and AD might be expected to shed light on these crucial events.

1. Johansson, S. G. *et al.* A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* **56**, 813–824 (2001).
This paper describes an overview of the history of atopic disease and its classification, and addresses many of the problems in understanding and classifying the incomplete overlap between atopy, asthma and AD.
2. von Pirquet, C. Allergie. *Münch med Wochenschr* **30**, 1457–1458 (1906).
3. Noon, L. Prophylactic innoculation against hay-fever. *Lancet* **i**, 1572–1573 (1911) .
4. Turner, H. & Kinet, J. P. Signalling through the high-affinity IgE receptor FcεRI. *Nature* **402**, B24–B30 (1999).
5. Kay, A. B. Allergy and allergic diseases. Second of two parts. *N. Engl. J. Med.* **344**, 109–113 (2001).
6. Kay, A. B. Allergy and allergic diseases. First of two parts. *N. Engl. J. Med.* **344**, 30–37 (2001).
References 5 and 6 give an excellent overview of current understanding of atopic diseases.
7. Crater, S. E. & Platts-Mills, T. A. Searching for the cause of the increase in asthma. *Curr. Opin. Pediatr.* **10**, 594–599 (1998).
8. Koenig, J. Air pollution and asthma. *J. Allergy Clin. Immunol.* **104**, 717–722 (1999).
9. Strachan, D. P. Hay fever, hygiene, and household size. *Brit. Med. J.* **299**, 1259–1260 (1989).
This paper is the first to use the term 'hygiene hypothesis'.
10. von Mutius, E. *et al.* Prevalence of asthma and atopy in two areas of West and East Germany. *Am. J. Respir. Crit. Care Med.* **149**, 358–364 (1994).
This landmark paper was the first to recognize that the prevalence of atopy is altered by the environment: in this case polluted East Germany contained less atopy than the unpolluted West.
11. Holt, P. G., Macaubas, C., Stumbles, P. A. & Sly, P. D. The role of allergy in the development of asthma. *Nature* **402**, B12–B17 (1999).
12. Holt, P. G. A potential vaccine strategy for asthma and allied atopic diseases during early childhood. *Lancet* **344**, 456–458 (1994).
This is part of a series of influential papers indicating that the T_H1–T_H2 balance could be altered by the neonatal environment.
13. Romagnani, S. The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? *Immunology* **112**, 352–363 (2004).
14. Cox, H. E. *et al.* Association of atopic dermatitis to the beta subunit of the high affinity immunoglobulin E receptor. *Br. J. Dermatol.* **138**, 182–187 (1998).
15. Creticos, P. S. *et al.* Ragweed immunotherapy in adult asthma. *N. Engl. J. Med.* **334**, 501–506 (1996).
16. Adkinson, N. F. Jr *et al.* A controlled trial of immunotherapy for asthma in allergic children. *N. Engl. J. Med.* **336**, 324–331 (1997).
17. Barnes, P. J. Is immunotherapy for asthma worthwhile? *N. Engl. J. Med.* **334**, 531–532 (1996).
18. Holgate, S. T. *et al.* Efficacy and safety of a recombinant anti-immunoglobulin E antibody (omalizumab) in severe allergic asthma. *Clin. Exp. Allergy* **34**, 632–638 (2004).
19. Farrall, M. Quantitative genetic variation: a post-modern view. *Hum. Mol. Genet.* **13**, R1–R7 (2004).
20. Duffy, D. L., Martin, N. G., Battistutta, D., Hopper, J. L. & Mathews, J. D. Genetics of asthma and hay fever in Australian twins. *Am. Rev. Respir. Dis.* **142**, 1351–1358 (1990).
21. Larsen, F. S., Holm, N. V. & Henningsen, K. Atopic dermatitis. A genetic-epidemiologic study in a population-based twin sample. *J. Am. Acad. Dermatol.* **15**, 487–494 (1986).
22. Schultz Larsen, F. Atopic dermatitis: a genetic-epidemiologic study in a population-based twin sample. *J. Am. Acad. Dermatol.* **28**, 719–723 (1993).
23. Cookson, W. A new gene for asthma: would you ADAM and Eve it? *Trends Genet.* **19**, 169–172 (2003).
24. Wills-Karp, M. & Ewart, S. L. Time to draw breath: asthma-susceptibility genes are identified. *Nature Rev. Genet.* **5**, 376–387 (2004).
This paper contains a comprehensive review of the current state of asthma genetics, including reference to murine models of disease.
25. Becker, K. *et al.* Clustering of non-major histocompatibility complex susceptibility candidate loci in human autoimmune diseases. *Proc. Natl Acad. Sci. USA* **95**, 9979–9984 (1998).
This paper was the first to observe clustering of inflammatory disease loci in a limited number of chromosomal segments.
26. Cookson, W. Genetics and genomics of asthma and allergic diseases. *Immunol. Rev.* **190**, 195–206 (2002).
27. Bowcock, A. M. & Cookson, W. O. The genetics of psoriasis, psoriatic arthritis and atopic dermatitis. *Hum. Mol. Genet.* **13**, R43–55 (2004).
This paper contains a review of the genetics of AD.
28. Tosh, K. *et al.* A region of chromosome 20 is linked to leprosy susceptibility in a South Indian population. *J. Infect. Dis.* **186**, 1190–1193 (2002).
29. Judge, M. R., Morgan, G. & Harper, J. I. A clinical and immunological study of Netherton's syndrome. *Br. J. Dermatol.* **131**, 615–621 (1994).
30. Bitoun, E. *et al.* Netherton syndrome: disease expression and spectrum of SPINK5 mutations in 21 families. *J. Invest. Dermatol.* **118**, 352–361 (2002).
31. Chavanas, S. *et al.* Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. *Nature Genet.* **25**, 141–142 (2000).
32. Mägert, H. J. *et al.* LEKTI, a novel 15-domain type of human serine proteinase inhibitor. *J. Biol. Chem.* **274**, 21499–21502 (1999).
33. Walley, A. J. *et al.* Gene polymorphism in Netherton and common atopic disease. *Nature Genet.* **29**, 175–178 (2001).
34. Nishio, Y. *et al.* Association between polymorphisms in the *SPINK5* gene and atopic dermatitis in the Japanese. *Genes Immun.* **4**, 515–517 (2003).
35. Kato, A. *et al.* Association of *SPINK5* gene polymorphisms with atopic dermatitis in the Japanese population. *Br. J. Dermatol.* **148**, 665–669 (2003).
36. Kabesch, M., Carr, D., Weiland, S. K. & von Mutius, E. Association between polymorphisms in serine protease inhibitor, kazal type 5 and asthma phenotypes in a large German population sample. *Clin. Exp. Allergy* **34**, 340–345 (2004).
37. Komatsu, N. *et al.* Elevated stratum corneum hydrolytic activity in Netherton syndrome suggests an inhibitory regulation of desquamation by SPINK5-derived peptides. *J. Invest. Dermatol.* **118**, 436–443 (2002).
38. Leyden, J. J., Marples, R. R. & Kligman, A. M. *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br. J. Dermatol.* **90**, 525–530 (1974).

39. Christophers, E. & Henseler, T. Contrasting disease patterns in psoriasis and atopic dermatitis. *Arch. Dermatol. Res.* **279** (Suppl.), S48–S51 (1987).
40. Miedzobrodzki, J., Kaszycki, P., Bialecka, A. & Kasprowicz, A. Proteolytic activity of *Staphylococcus aureus* strains isolated from the colonized skin of patients with acute-phase atopic dermatitis. *Eur. J. Clin. Microbiol. Infect. Dis.* **21**, 269–276 (2002).
This paper shows that the *S. aureus* that are associated with AD have high levels of protease activity.
41. Winton, H. L. *et al.* Class specific inhibition of house dust mite proteinases which cleave cell adhesion, induce cell death and which increase the permeability of lung epithelium. *Br. J. Pharmacol.* **124**, 1048–1059 (1998).
42. Wachter, A. M. & Lezdey, J. Treatment of atopic dermatitis with α1-proteinase inhibitor. *Ann. Allergy* **69**, 407–414 (1992).
43. Cookson, W. O. *et al.* Genetic linkage of childhood atopic dermatitis to psoriasis susceptibility loci. *Nature Genet.* **27**, 372–373 (2001).
44. Capon, F. *et al.* Searching for psoriasis susceptibility genes in Italy: genome scan and evidence for a new locus on chromosome 1. *J. Invest. Dermatol.* **112**, 32–35 (1999).
45. Mischke, D., Korge, B. P., Marenholz, I., Volz, A. & Ziegler, A. Genes encoding structural proteins of epidermal cornification and S100 calcium-binding proteins form a gene complex ('epidermal differentiation complex') on human chromosome 1q21. *J. Invest. Dermatol.* **106**, 989–992 (1996).
46. Nomura, I. *et al.* Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *J. Allergy Clin. Immunol.* **112**, 1195–1202 (2003).
47. Bowcock, A. M. *et al.* Insights into psoriasis and other inflammatory diseases from large-scale gene expression studies. *Hum. Mol. Genet.* **10**, 1793–1805 (2001).
48. Zhou, X. *et al.* Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. *Physiol. Genomics* **13**, 69–78 (2003).
49. Marshall, D., Hardman, M. J., Nield, K. M. & Byrne, C. Differentially expressed late constituents of the epidermal cornified envelope. *Proc. Natl Acad. Sci. USA* **98**, 13031–13036 (2001).
50. Lohman, F. *et al.* Expression of the SPRR cornification genes is differentially affected by carcinogenic transformation. *Exp. Cell Res.* **231**, 141–148 (1997).
51. Hardas, B. *et al.* Assignment of psoriasin to human chromosomal band 1q21: coordinate overexpression of clustered genes in psoriasis. *J. Invest. Dermatol.* **106**, 753–758 (1996).
52. Christiano, A. M. Frontiers in keratodermas: pushing the envelope. *Trends Genet.* **13**, 227–233 (1997).
53. Donato, R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. *Int. J. Biochem. Cell Biol.* **33**, 637–668 (2001).
54. Allen, M. *et al.* Positional cloning of a novel gene influencing asthma from chromosome 2q14. *Nature Genet.* **35**, 258–263 (2003).
55. Laitinen, T. *et al.* Characterization of a common susceptibility locus for asthma-related traits. *Science* **304**, 300–304 (2004).
56. Zhang, Y. *et al.* Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. *Nature Genet.* **34**, 181–186 (2003).
57. Van Eerdewegh, P. *et al.* Association of the *ADAM33* gene with asthma and bronchial hyperresponsiveness. *Nature* **418**, 426–430 (2002).
58. Kuperman, D. A. *et al.* Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nature Med.* **8**, 885–889 (2002).
59. Ober, C. *et al.* Variation in the interleukin 4-receptor alpha gene confers susceptibility to asthma and atopy in ethnically diverse populations. *Am. J. Hum. Genet.* **66**, 517–526 (2000).
60. Donnadieu, E. *et al.* Competing functions encoded in the allergy-associated FcεRIβ gene. *Immunity* **18**, 665–674 (2003).
61. Traherne, J. A. *et al.* LD mapping of maternally and non-maternally derived alleles and atopy in FcεRI-β. *Hum. Mol. Genet.* **12**, 2577–2585 (2003).
62. Oguma, T. *et al.* Role of prostanoid DP receptor variants in susceptibility to asthma. *N. Engl. J. Med.* **351**, 1752–1763 (2004).
63. Baldini, M. *et al.* A polymorphism* in the 5′ flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am. J. Respir. Cell Mol. Biol.* **20**, 976–983 (1999).
64. Eder, W. *et al.* Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J. Allergy Clin. Immunol.* **113**, 482–488 (2004).
65. Kabesch, M. *et al.* Association between polymorphisms in caspase recruitment domain containing protein 15 and allergy in two German populations. *J. Allergy Clin. Immunol.* **111**, 813–817 (2003).
66. McIntire, J. J. *et al.* Immunology: hepatitis A virus link to atopic disease. *Nature* **425**, 576 (2003).
67. Raby, B. A. *et al.* Polymorphisms in toll-like receptor 4 are not associated with asthma or atopy-related phenotypes. *Am. J. Respir. Crit. Care Med.* **166**, 1449–1456 (2002).
68. Noguchi, E. *et al.* An association study of asthma and total serum immunoglobin E levels for Toll-like receptor polymorphisms in a Japanese population. *Clin. Exp. Allergy* **34**, 177–183 (2004).
69. Lazarus, R. *et al.* TOLL-like receptor 10 genetic variation is associated with asthma in two independent samples. *Am. J. Respir. Crit. Care Med.* **170**, 594–600 (2004).
70. Moffatt, M. F. & Cookson, W. O. Tumour necrosis factor haplotypes and asthma. *Hum. Mol. Genet.* **6**, 551–554 (1997).
71. Pulleyn, L. J., Newton, R., Adcock, I. M. & Barnes, P. J. TGFβ1 allele association with asthma severity. *Hum. Genet.* **109**, 623–627 (2001).
72. Hewett, D. *et al.* Identification of a psoriasis susceptibility candidate gene by linkage disequilibrium mapping with a localized single nucleotide polymorphism map. *Genomics* **79**, 305–314 (2002).
73. Tiilikainen, A., Lassus, A., Karvonen, J., Vartiainen, P. & Julin, M. Psoriasis and HLA-Cw6. *Brit. J. Dermatol.* **102**, 179–184 (1980).
74. Ahnini, R. T. *et al.* Novel genetic association between the corneodesmosin (*MHC S*) gene and susceptibility to psoriasis. *Hum. Mol. Genet.* **8**, 1135–1140 (1999).
75. Asumalahti, K. *et al.* A candidate gene for psoriasis near HLA-C, HCR (Pg8), is highly polymorphic with a disease-associated susceptibility allele. *Hum. Mol. Genet.* **9**, 1533–1542 (2000).
76. Allen, M. *et al.* Corneodesmosin expression in psoriasis vulgaris differs from normal skin and other inflammatory skin disorders. *Lab. Invest.* **81**, 969–976 (2001).
77. Ogura, Y. *et al.* A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* **411**, 603–606 (2001).
78. Hugot, J. P. *et al.* Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* **411**, 599–603 (2001).
79. Stoll, M. *et al.* Genetic variation in DLG5 is associated with inflammatory bowel disease. *Nature Genet.* **36**, 476–480 (2004).
80. Peltekova, V. D. *et al.* Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nature Genet.* **36**, 471–475 (2004).
81. Lala, S. *et al.* Crohn's disease and the NOD2 gene: a role for paneth cells. *Gastroenterology* **125**, 47–57 (2003).
82. Hisamatsu, T. *et al.* CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. *Gastroenterology* **124**, 993–1000 (2003).
83. Kamada, F. *et al.* Association of the *hCLCA1* gene with childhood and adult asthma. *Genes Immun.* (2004).
84. Hegab, A. E. *et al. CLCA1* gene polymorphisms in chronic obstructive pulmonary disease. *J. Med. Genet.* **41**, e27 (2004).
85. Tomic-Canic, M., Komine, M., Freedberg, I. M. & Blumenberg, M. Epidermal signal transduction and transcription factor activation in activated keratinocytes. *J. Dermatol. Sci.* **17**, 167–181 (1998).
86. Freedberg, I., Tomic-Canic, M., Komine, M. & Blumenberg, M. Keratins and the keratinocyte activation cycle. *J. Invest. Dermatol.* **116**, 633–640 (2001).
87. Song, P. I. *et al.* Human keratinocytes express functional CD14 and Toll-like receptor 4. *J. Invest. Dermatol.* **119**, 424–432 (2002).
88. Muir, A. *et al.* Toll-like receptors in normal and cystic fibrosis airway epithelial cells. *Am. J. Respir. Cell Mol. Biol.* **30**, 777–783 (2004).
89. Ichikawa, J. K. *et al.* Interaction of pseudomonas aeruginosa with epithelial cells: identification of differentially regulated genes by expression microarray analysis of human cDNAs. *Proc. Natl Acad. Sci. USA* **97**, 9659–9664 (2000).
90. Belcher, C. E. *et al.* The transcriptional responses of respiratory epithelial cells to *Bordetella pertussis* reveal host defensive and pathogen counter-defensive strategies. *Proc. Natl Acad. Sci. USA* **97**, 13847–13852 (2000).
91. Clauss, A., Lilja, H. & Lundwall, A. A locus on human chromosome 20 contains several genes expressing protease inhibitor domains with homology to whey acidic protein. *Biochem. J.* **368**, 233–242 (2002).
92. Simpson, A. J., Maxwell, A. I., Govan, J. R., Haslett, C. & Sallenave, J. M. Elafin (elastase-specific inhibitor) has anti-microbial activity against gram-positive and gram-negative respiratory pathogens. *FEBS Lett.* **452**, 309–313 (1999).
93. Singh, P. K., Tack, B. F., McCray, P. B. Jr & Welsh, M. J. Synergistic and additive killing by antimicrobial factors found in human airway surface liquid. *Am. J. Physiol. Lung. Cell. Mol. Physiol.* **279**, L799–805 (2000).
94. Tomee, J. F., Koeter, G. H., Hiemstra, P. S. & Kauffman, H. F. Secretory leukoprotease inhibitor: a native antimicrobial protein presenting a new therapeutic option? *Thorax* **53**, 114–116 (1998).
95. Bals, R. *et al.* Human β-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung. *J. Clin. Invest.* **102**, 874–880 (1998).
96. Bals, R., Wang, X., Zasloff, M. & Wilson, J. M. The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. *Proc. Natl Acad. Sci. USA* **95**, 9541–9546 (1998).
97. Huttner, K. M. & Bevins, C. L. Antimicrobial peptides as mediators of epithelial host defense. *Pediatr. Res.* **45**, 785–794 (1999).
98. Brogan, T. D., Ryley, H. C., Neale, L. & Yassa, J. Soluble proteins of bronchopulmonary secretions from patients with cystic fibrosis, asthma, and bronchitis. *Thorax* **30**, 72–79 (1975).
99. Benson, M. *et al.* DNA microarrays to study gene expression in allergic airways. *Clin. Exp. Allergy* **32**, 301–308 (2002).
100. Kaufman, H. S. & Hobbs, J. R. Immunoglobulin deficiencies in an atopic population. *Lancet* **2**, 1061–1063 (1970).
101. Ludviksson, B. R., Eiriksson, T. H., Ardal, B., Sigfusson, A. & Valdimarsson, H. Correlation between serum immunoglobulin A concentrations and allergic manifestations in infants. *J. Pediatr.* **121**, 23–27 (1992).
102. Gallucci, S., Lolkema, M. & Matzinger, P. Natural adjuvants: endogenous activators of dendritic cells. *Nature Med.* **5**, 1249–55 (1999).
103. Matzinger, P. The danger model: a renewed sense of self. *Science* **296**, 301–305 (2002).
Matzinger explains that danger signals are essential for the initiation of an immune response.
104. Palaniyar, N., Nadesalingam, J. & Reid, K. B. Pulmonary innate immune proteins and receptors that interact with gram-positive bacterial ligands. *Immunobiology* **205**, 575–594 (2002).
105. Kopp, E. & Medzhitov, R. Recognition of microbial infection by Toll-like receptors. *Curr. Opin. Immunol.* **15**, 396–401 (2003).
106. Inohara, N. & Nunez, G. NODS: intracellular proteins involved in inflammation and apoptosis. *Nature Rev. Immunol.* **3**, 371–382 (2003).
107. Chamaillard, M., Girardin, S. E., Viala, J. & Philpott, D. J. Nods, Nalps and Naip: intracellular regulators of bacterial-induced inflammation. *Cell. Microbiol.* **5**, 581–592 (2003).
108. Seong, S. Y. & Matzinger, P. Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. *Nature Rev. Immunol.* **4**, 469–478 (2004).
109. Ring, P. C. *et al.* The 18-kDa form of cat allergen *Felis domesticus* 1 (*Fel d* 1) is associated with gelatin- and fibronectin-degrading activity. *Clin. Exp. Allergy* **30**, 1085–1096 (2000).
110. Bufe, A., Schramm, G., Keown, M. B., Schlaak, M. & Becker, W. M. Major allergen *Phl p* Vb in timothy grass is a novel pollen RNase. *FEBS Lett.* **363**, 6–12 (1995).
111. Tjabringa, G. S. *et al.* The antimicrobial peptide LL-37 activates innate immunity at the airway epithelial surface by transactivation of the epidermal growth factor receptor. *J. Immunol.* **171**, 6690–6696 (2003).
112. Yang, D., Biragyn, A., Kwak, L. W. & Oppenheim, J. J. Mammalian defensins in immunity: more than just microbicidal. *Trends Immunol.* **23**, 291–296 (2002).
113. Yoshio, H. *et al.* Antimicrobial polypeptides of human vernix caseosa and amniotic fluid: implications for newborn innate defense. *Pediatr. Res.* **53**, 211–216 (2003).
114. Brandtzaeg, P. *et al.* The leucocyte protein L1 (calprotectin): a putative nonspecific defence factor at epithelial surfaces. *Adv. Exp. Med. Biol.* **371A**, 201–206 (1995).
115. Steinbakk, M. *et al.* Antimicrobial actions of calcium binding leucocyte L1 protein, calprotectin. *Lancet* **336**, 763–765 (1990).
116. Miyasaki, K. T., Bodeau, A. L., Murthy, A. R. & Lehrer, R. I. *In vitro* antimicrobial activity of the human neutrophil cytosolic S-100 protein complex, calprotectin, against Capnocytophaga sputigena. *J. Dent. Res.* **72**, 517–523 (1993).
117. Gottsch, J. D., Eisinger, S. W., Liu, S. H. & Scott, A. L. Calgranulin C has filariacidal and filariastatic activity. *Infect. Immun.* **67**, 6631–6636 (1999).
118. Yang, D. *et al.* Many chemokines including CCL20/MIP-3α display antimicrobial activity. *J. Leukoc. Biol.* **74**, 448–455 (2003).
A review highlighting that many chemokines have antimicrobial activity.

119. Pulendran, B., Palucka, K. & Banchereau, J. Sensing pathogens and tuning immune responses. *Science* **293**, 253–256 (2001).
A good review of how specific pathogen recognition alters the nature of the immune response.
120. Stumbles, P. A. *et al.* Resting respiratory tract dendritic cells preferentially stimulate T helper cell type 2 (T_H2) responses and require obligatory cytokine signals for induction of T_H1 immunity. *J. Exp. Med.* **188**, 2019–2031 (1998).
121. Mazzoni, A. & Segal, D. M. Controlling the Toll road to dendritic cell polarization. *J. Leukoc. Biol.* **75**, 721–730 (2004).
122. Rook, G. A. & Stanford, J. L. Give us this day our daily germs. *Immunol. Today* **19**, 113–116 (1998).
123. Bjorksten, B. Effects of intestinal microflora and the environment on the development of asthma and allergy. *Springer Semin. Immunopathol.* **25**, 257–270 (2004).
124. Hooper, L. V. & Gordon, J. I. Commensal host-bacterial relationships in the gut. *Science* **292**, 1115–1118 (2001).
125. Sepp, E. *et al.* Intestinal microflora of Estonian and Swedish infants. *Acta Paediatr.* **86**, 956–961 (1997).
126. Bjorksten, B., Naaber, P., Sepp, E. & Mikelsaar, M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clin. Exp. Allergy* **29**, 342–346 (1999).
127. Svanes, C., Jarvis, D., Chinn, S. & Burney, P. Childhood environment and adult atopy: results from the European Community Respiratory Health Survey. *J. Allergy Clin. Immunol.* **103**, 415–420 (1999).
128. Hesselmar, B., Aberg, N., Aberg, B., Eriksson, B. & Bjorksten, B. Does early exposure to cat or dog protect against later allergy development? *Clin. Exp. Allergy* **29**, 611–617 (1999).
129. von Mutius, E. *et al.* Skin test reactivity and number of siblings. *BMJ* **308**, 692–695 (1994).
130. Riedler, J. *et al.* Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* **358**, 1129–1133 (2001).
This paper describes the strongly protective effect of farm environments and non-pasteurized milk on the prevalence of asthma in Alpine regions.
131. Wickens, K. *et al.* Farm residence and exposures and the risk of allergic diseases in New Zealand children. *Allergy* **57**, 1171–1179 (2002).
132. Komada, T. *et al.* Novel specific chemtactic receptor for S100L protein on guinea pig eosinophils. *Biochem. Biophys. Res. Commun.* **220**, 871–874 (1996).
133. Jinquan, T. *et al.* Psoriasin: a novel chemotactic protein. *J. Invest. Dermatol.* **107**, 5–10 (1996).
134. Passey, R. J., Xu, K., Hume, D. A. & Geczy, C. L. S100A8: emerging functions and regulation. *J. Leukoc. Biol.* **66**, 549–556 (1999).
135. Lackmann, M. *et al.* Identification of a chemotactic domain of the pro-inflammatory S100 protein CP-10. *J. Immunol.* **150**, 2981–2991 (1993).
136. Cornish, C. J. *et al.* S100 protein CP-10 stimulates myeloid cell chemotaxis without activation. *J. Cell. Physiol.* **166**, 427–437 (1996).
137. Eue, I., Pietz, B., Storck, J., Klempt, M. & Sorg, C. Transendothelial migration of 27E10$^+$ human monocytes. *Int. Immunol.* **12**, 1593–1604 (2000).
138. Yui, S., Mikami, M. & Yamazaki, M. Purification and characterization of the cytotoxic factor in rat peritoneal exudate cells: its identification as the calcium binding protein complex, calprotectin. *J. Leukoc. Biol.* **58**, 307–316 (1995).
139. Aguiar-Passeti, T., Postol, E., Sorg, C. & Mariano, M. Epithelioid cells from foreign-body granuloma selectively express the calcium-binding protein MRP-14, a novel down-regulatory molecule of macrophage activation. *J. Leukoc. Biol.* **62**, 852–858 (1997).
140. Brun, J. G., Ulvestad, E., Fagerhol, M. K. & Jonsson, R. Effects of human calprotectin (L1) on *in vitro* immunoglobulin synthesis. *Scand. J. Immunol.* **40**, 675–680 (1994).
141. Hofmann, M. A. *et al.* RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell* **97**, 889–901 (1999).

Competing interests statement
The authors declare no competing financial interests.

Online links

DATABASES
The following terms in this article are linked online to:
Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
ADAM33 | *CD14* | *CLCA1* | *DLG5* | *DPP10* | *FCERI* | IL-4 | IL-5 | IL-9 | IL-13 | *NOD2* | *PHF11* | *PSORS1* | *S100A12* | *SLC12A8* | *SLC22A4* | *SLC22A5* | *SLC9A3R1* | SPINK5 | *SPRR2C* | *TGFB* | *TIM1* | *TLR2* | *TLR10*
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
ankylosing spondylitis | multiple sclerosis | Netherton's disease | rheumatoid arthritis | type 1 diabetes
Access to this interactive links box is free online.