NEW TARGETS FOR ALLERGIC RHINITIS — A DISEASE OF CIVILIZATION

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Allergic rhinitis is an inflammatory disorder of the nasal mucosa, mediated by T_H2 lymphocytes, which is linked to atopy and whose prevalence is increasing in association with a Western lifestyle. The production of allergen-specific IgE, activation of mucosal mast cells and the recruitment and activation of effector leukocytes provides potential therapeutic targets, including selective inhibition of cytokines, adhesion molecules and signalling pathways. Blockade of IgE, using monoclonal antibodies and vaccine strategies, is a new approach for interrupting the allergic cascade, whereas the use of recombinant mutated allergens, peptides and DNA oligonucleotides will lead to improved efficacy and reduced side effects of immunotherapy to induce tolerance.

DESENSITIZE
The use of allergens or modified allergens to induce immunological tolerance.

CHROMONES
Anti-allergic drugs originally derived from *Rhellin* from the plant *Amni visnaga*.

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Allergic rhinitis (hayfever) is a common clinical condition that affects approximately 30% of adults and up to 40% of children in industrialized societies[1]. Seasonal allergic rhinitis is triggered by prevailing tree, grass or weed pollens in the spring and summer months, whereas the more chronic perennial allergic rhinitis involves indoor allergens, usually from dust mites, animals and fungi (FIG. 1). The economic toll of allergic rhinitis is evident from studies investigating both direct costs of medications (for example, in 2002 US $6 billion spent on prescription medications)[2], as well as indirect costs (such as the 3.5 million lost workdays and 2 million missed school days annually in the United States)[3]. The current understanding of disease pathogenesis (FIG. 2) has led to the current treatment options for allergic rhinitis, which include allergen avoidance (whenever practical), anti-allergic medications (oral or intranasal) and immunotherapy to DESENSITIZE patients to specific allergens. Available medications include antihistamines (H_1 histamine receptor antagonists), decongestants, leukotriene inhibitors, topical CHROMONES and corticosteroids. Although presently available therapies — singly or in combination — control symptoms in the majority of patients' rhinitis, there continues to be those with moderate–severe disease who remain symptomatic despite conventional treatment[4].

Gene–environmental interaction

There is a strong genetic as well as environmental component to the allergic inflammatory response, which is evident from genome-wide screens that have identified chromosomal regions of linkage to asthma, allergen-specific immunoglobulin E (IgE) production (ATOPY) and total IgE levels[5]. Susceptibility genes specific for allergic rhinitis have not been as well characterized as those for atopy and asthma, but are known to include major histocompatibility complex (MHC) alleles on chromosome 6 that are involved in antigen recognition. Although a genetic susceptibility to allergic rhinitis is important, interactions with the appropriate environment is crucial for the emergence of atopy and its expression as rhinitis, especially early-life exposure to microorganisms, which is sometimes referred to as the hygiene hypothesis (FIG. 3). One example of how exposure to microorganisms early in life can alter the natural history of allergic disease is the finding that children who are raised on farms with livestock show a 50–70% reduction in atopy, an observation that has been attributed to bacterial endotoxin exposure producing immunological programming via Toll-like receptor-4 (TLR4)[6]. Other observations cited in favour of the hygiene hypothesis include the protective effects exerted by older siblings in a family, which could possibly result from the early

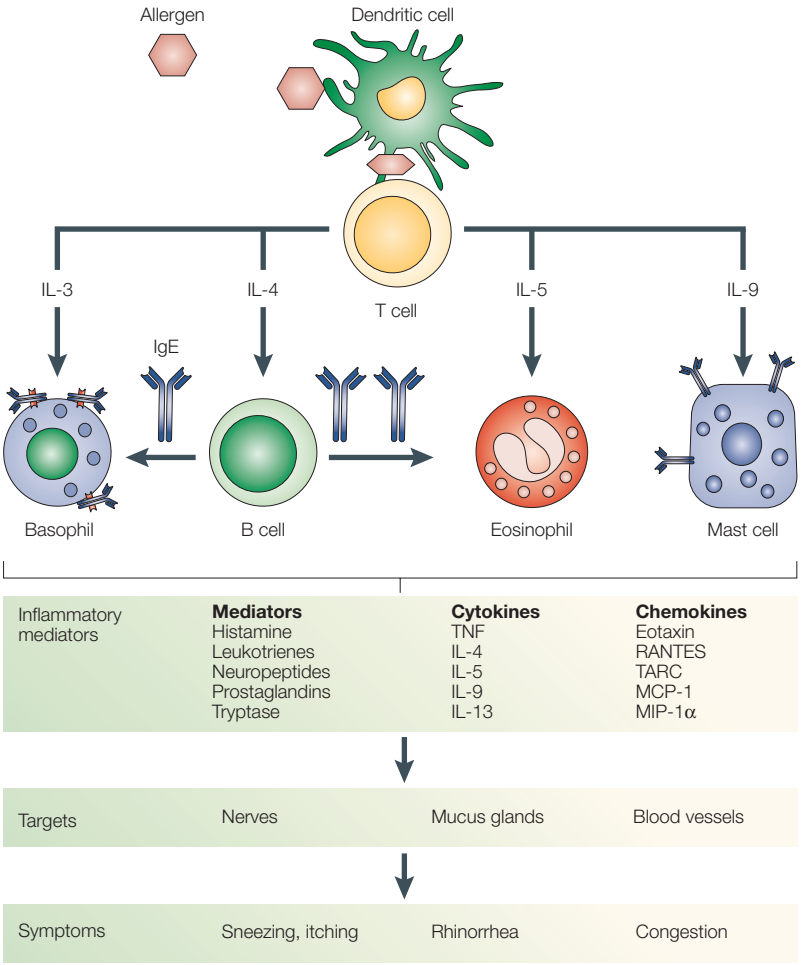

Figure 1 | **Cell and mediator pathways underlying the pathogenesis of allergic rhinitis.** Allergic rhinitis is an inflammatory disease of the upper airways that is characterized by the development of symptoms including rhinorrhea, sneezing, nasal congestion and itching of the palate. These symptoms result from activation of resident and recruited inflammatory cells in the nasal mucosa, afferent nerve stimulation, glandular hypersecretion and increased vascular permeability. Ig, immunoglobulin; IL, interleukin; MCP, monocyte chemoattractant protein (also known as CCL2); MIP, macrophage inflammatory protein (also known as CCL3); RANTES, regulated on activation, normal T-cell expressed and secreted; TARC, thymus and activation-regulated chemokine (also known as CCL17); TNF, tumour-necrosis factor.

exposure of younger siblings to common cold viruses and gastrointestinal infections including *Helicobacter pylori* and hepatitis A[7] (FIG. 3). TLR9 has been similarly linked to protection afforded by non-methylated CpG DNA oligonucleotides (also known as immunostimulatory sequences (ISSs)) from bacteria. Clearly, such observations have important implications for the identification of new pathways that can be targeted to prevent and treat allergic diseases such as rhinitis.

When an allergen is inhaled in subjects with allergic rhinitis, antigen-presenting cells (APCs) in the nasal mucosa (especially dendritic cells) take up the allergen, process it and present it as small peptide fragments in conjunction with MHC class II molecules to allergen-specific T_H2 cells[6,8] that generate pro-allergic cytokines (FIG. 4). Subsequent inhalation of the same allergen results in crosslinking of allergen-specific IgE via the Fc_ε receptor $Fc_\varepsilon R1$ that is present on mast cells to release pre-formed granule mediators, such as histamine, heparin and tryptase, and generate lipid mediators, such as leukotriene C_4 (LTC_4), prostaglandin D_2 (PGD_2), as well as many inflammatory cytokines and chemokines (FIG. 2). Although exerting different effects in the nasal mucosa, many of the mediators are involved in the recruitment of secondary effector leukocytes (FIG. 5).

An improved understanding of the cellular and molecular mechanisms underlying the pathogenesis of allergic rhinitis has resulted in the identification of potential novel therapeutic strategies for its treatment. In theory, the inhibition of an upstream pathway in the allergic cascade (for example, the dendritic cells or T_H2 cells) is likely to make a greater clinical contribution, compared with the inhibition of a single downstream mediator. H_1 antihistamines are an example of a strategy directed at inhibiting a single mediator that is very effective in patients with mild to moderate allergic rhinitis. However, of the current drugs available, intranasal corticosteroids, which inhibit several pro-inflammatory pathways in allergic rhinitis, are more effective than single-mediator antagonists[9]. We will review the current status of novel therapeutic approaches to the treatment of allergic rhinitis, focusing on use of immunotherapy (both peptide- and DNA-based), as well as strategies to inhibit IgE, mast-cell activation, cytokines, inflammatory cell recruitment and specific mediators.

Mediator inhibitors

The new generation of H_1 antihistamines are usually metabolites of the older generation of drugs, and show greatly improved efficacy and safety because they act as inverse agonists[10]. A number of antihistamines are also claimed to exert anti-inflammatory actions, but at therapeutic concentrations the clinical significance of this is questionable[11]. In addition to H_1, H_2 and H_3 receptor subtypes, the recent discovery of the H_4 receptor expressed on mast cells, basophils and eosinophils has generated renewed interest in histamine, because H_4 receptors mediate Ca^{2+} signalling and chemotaxis[12]. It is possible that selective H_4 antagonists could have anti-inflammatory actions in allergic disease. Cysteinyl leukotrienes (CystLTs) are also AUTACOID mediators released from mast cells, basophils, eosinophils and macrophages and are particularly important in causing nasal blockage[13]. Clinical trials of the $CystLT_1$ receptor antagonists montelukast and zafirlukast in allergic rhinitis demonstrated their efficacy[14,15], but this is not improved by the addition of an H_1 antagonist[16], and overall is less than that achieved with nasal corticosteroids[9]. The recent discovery of both $CystLT_1$ and LT_2 receptors on eosinophils, which differ in their avidity for the leukotrienes LTD_4 and LTC_4, indicates a potential pro-inflammatory role for this mediator class[17] that could be usefully inhibited with a dual antagonist. PGD_2 is another mast-cell-derived EICOSANOID with potent vasodilator properties, which are mediated by the DP_1 receptor (C.A. Rizz, personal communication). DP_1 receptor antagonists offer promise for situations in which nasal blockage is problematic (G. P. O'Neill, personal communication). A second PGD_2 receptor, DP_2 (also known as CRTH2), has been

ATOPY
The genetic susceptibility to produce IgE antibodies against common environmental allergens.

CpG DNA
Also known as immunostimulatory sequences (ISS), these are sequence-specific non-methylated DNA molecules of cytosine and guanosine, modelled on those present in microorganisms, which have the capacity to stimulate Toll-like receptor-9 found on antigen-presenting cells and to modify immune responses.

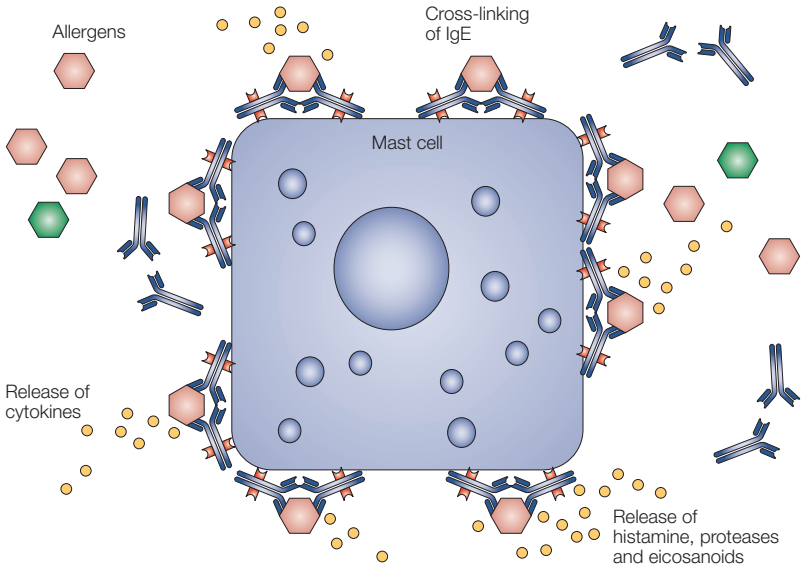

Figure 2 | **Interaction between allergens and IgE to activate mast cells via the high-affinity receptor $Fc_{\varepsilon}RI$.** Airborne allergens including pollens, moulds, dust mites and animal dander can provoke rhinitis symptoms in individuals sensitized to a particular allergen as a result of natural environmental allergen exposure, or following nasal allergen challenge in a research laboratory. Initiation of the response occurs through the crosslinking of immunoglobulin E (IgE) bound to high-affinity receptors ($Fc_{\varepsilon}R1$) on mucosal mast cells. This causes the release an array of mediators and cytokines with the capacity to cause an immediate response, as well as more delayed effects through the recruitment and activation of secondary leukocytes including eosinophils and basophils.

identified as a T_H2 marker (A. N. Hata, personal communication), but is also expressed on eosinophils and basophils, where it serves a chemotactic function, antagonism of which could have anti-inflammatory effects[18,19].

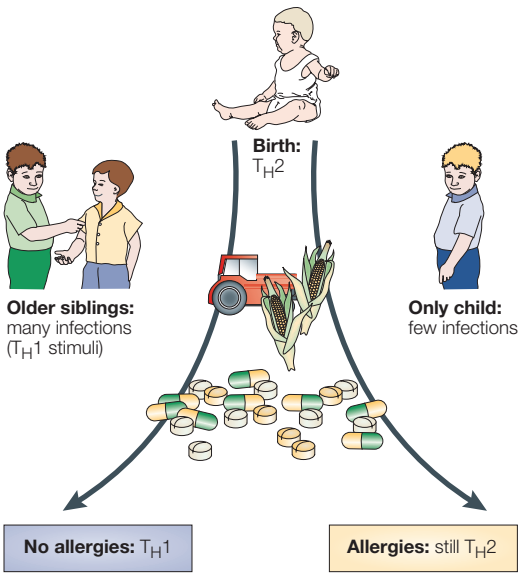

Figure 3 | **Schematic representation of the hygiene hypothesis.** Epidemiological studies have shown that family size, socio-economic status, diet and exposure to inhaled and ingested microorganisms (or factors derived from them) all influence the development of allergic sensitization. It is proposed that the worldwide rising trends in allergic disease is in some way linked to the adoption of the modern Westernized lifestyle, a proposition referred to as the hygiene hypothesis[9].

AUTACOID
Low-molecular-weight mediators that participate in cell–cell communication.

EICOSANOID
Mediators derived from polysaturated fatty acids.

LEUKOCYTE IMMUNOGLOBULIN-LIKE RECEPTORS
Cell-surface molecules whose activation leads to the phosphorylation of immunoreceptor tyrosine-based motifs (ITIMS) on adjacent receptors such as $Fc_{\varepsilon}R1$.

Mast-cell granules contain high concentrations of the protease tryptase. Three human mast-cell tryptases have been identified: α-, β- and γ-forms, the β-form being most abundant and active[20]. Recently, a membrane-anchored (γ)-tryptase has been described that differs from the other forms of this enzyme in not self-assembling into a tetramer[21]. Tryptase exerts a range of inflammatory responses that have been implicated in chronic tissue injury and remodelling, which possibly involve coagulation Factor II receptor-like 1 (F2RL1, also known as protease-activated receptor-2 (PAR2)) found on epithelial cells, fibroblasts and smooth muscle. A number of tryptase inhibitors have been described and some efficacy in humans has been reported in allergic models, including allergen challenge[22] (Y. Kato, personal communication).

Mast-cell stabilizers

Sodium cromoglycate and its successor nedocromil sodium are thought to act as mast-cell stabilizers, but their precise mechanism(s) of action is not known. A clearer understanding of activation–secretion coupling involving IgE and other receptors has opened up new possibilities for inhibiting mast-cell mediator release with greater efficacy than achievable with the cromones[23]. The finding that genistein, a potent inhibitor of tyrosine kinase, has potent anti-inflammatory activity on the mast-cell-dependent early- and late-phase allergen-provoked inflammatory reaction in the airways of guinea pigs provides proof of concept for the efficacy of selective inhibitors of protein kinases linked to mast-cell activation[24]. Mast cells also express receptors that are able to inhibit IgE-dependent degranulation through the activation of immuno-receptor tyrosine-based inhibitory motifs (ITIMS). On associating with the $Fc_{\varepsilon}R1$, inhibitory receptors, such as immunoglobulin-like transcripts (ILTs) and LEUKOCYTE IMMUNOGLOBULIN-LIKE RECEPTORS (LIRs), are able to affect IgE signalling by triggering phosphorylation of ITIM sequences on the γ-chains of $Fc_{\varepsilon}R1$. At present, 13 LIRs are recognized, and LIRs 1, 2, 3, 5 and 8 have inhibitory effects. LIR5 (gp49A and gp49B) is expressed at a high level by mast cells[25]. Co-ligation of gp49B with $Fc_{\varepsilon}R1$ powerfully inhibits mast-cell mediator secretion by dephosphorylating key signalling molecules and inhibiting that intracellular calcium mobilization that is necessary for triggering mast-cell activation. In mice whose gene encoding gp49B has been disrupted, enhanced mast-cell responsiveness occurs, which is manifested as increased systemic and local allergic responses[26]. Although no natural ligands for these receptors have yet been identified, they offer great potential for directing novel inhibitory agents. In addition to a range of cytokines and growth factors, mast cells are dependent on stem-cell factor (SCF) for differentiation, survival and optimal secretion[27]. In disorders such as mastocytosis, blockade of SCF Src kinase activity by the selective inhibitor PP1 has a marked effect in suppressing mast-cell proliferation[28]. Ablation of mast cells in the nasal mucosa would clearly have a large benefit in allergic rhinitis, in which mucosal mast-cell

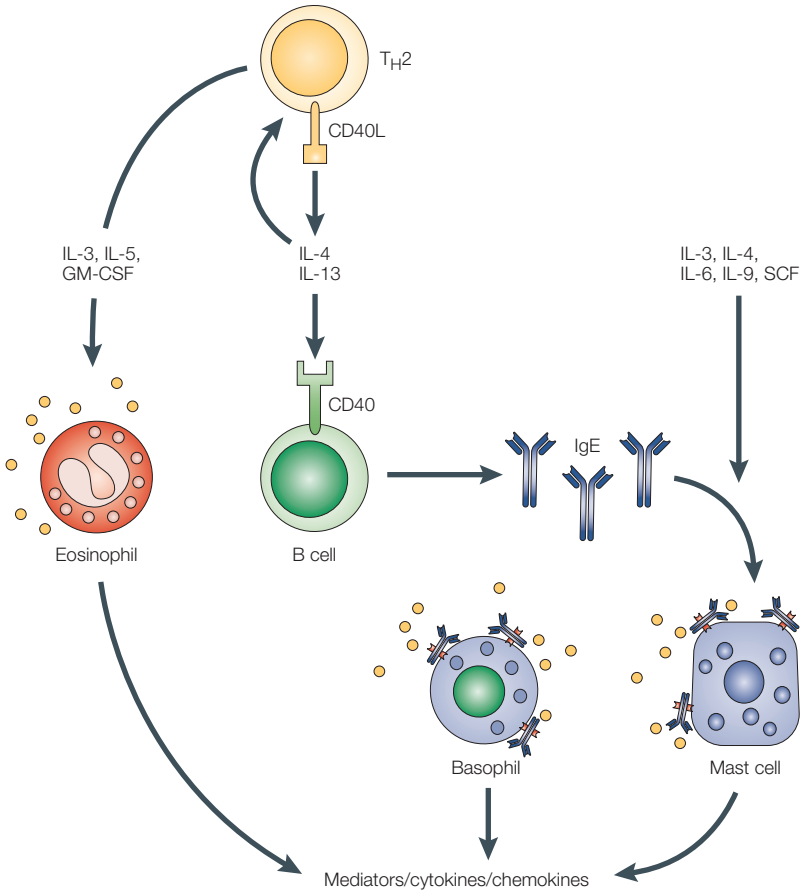

Figure 4 | **Central role of the T_H2 type lymphocyte in the orchestration of the chronic allergic phenotype.** The activated T_H2 cell produces cytokines that control immunoglobulin (Ig)E synthesis (for example, interleukin (IL)-4), mast cell (for example, IL-9), and eosinophil proliferation, maturation and priming (for example, IL-5). GM-CSF, granulocyte–macrophage colony-stimulating factor; SCF, stem cell factor.

populations markedly increase. Mast cells also express inwardly rectifying and Ca^{2+}-activated K^+ channels, and Ca^{2+}-independent Cl^- channels linked to IgE-dependent activation[29,30]. Selective blockade of these or other ion channels linked to activation–secretion coupling offer real promise for a new generation of anti-allergic drugs.

Inhibitors of neural pathways

In addition to inflammatory cells, allergic rhinitis is characterized by local neural activity, such as itching, sneezing and reflex-mediated secretion[31,32]. In allergic rhinitis, the topical application of substance P into the nasal mucosa increases eosinophil influx[33]; however, antagonists of the receptors for tachykinin precursor 1 and tachykinin 3 (also known as neurokinin-1 and –2, respectively) have so far proven disappointing when tested in clinical trials. For bradykinin, which is a potent releaser of neuropeptides, efficacy of a B_2 agonist has been reported in nasal allergen challenge[34], but subsequent Phase III clinical trials proved disappointing. Other neuropeptides also provide interesting targets, including calcitonin-gene-related peptide (CGRP) in chronic vasodilation[35]; and secretoneurin, which is present in cholinergic, adrenergic and sensory nerves[36] and which exerts a pro-inflammatory effect on eosinophils[37]. If suitable antagonists for these mediators are found then they are likely to be efficacious in the more chronic forms of allergic rhinitis or non-allergic vasomotor rhinitis, in which nasal blockage dominates.

Allergen-specific immunotherapy

The goal of allergen-specific immunotherapy (SIT) is to modulate the immune response to the administered allergen and thereby reduce the symptoms of allergic rhinitis. Recent advances in the molecular characterization of protein allergens has enabled standardization of the allergens used in allergen-specific immunotherapy, as well as the definition of an optimum dose range, of 6–24 μg of injected airborne allergen, to elicit a therapeutic response in patients with allergic rhinitis[38,39]. SIT is administered as a series of subcutaneous injections of highly purified airborne allergen(s) to patients with allergic rhinitis who are specifically sensitized to the allergen(s), as revealed by skin-prick testing or the presence of circulating allergen-specific IgE. SIT is clinically effective in reducing symptoms, as evidenced by the inhibition of both the allergen-provoked early- and late-phase nasal responses, reduced rhinitis symptoms, decreased risk of the subsequent development of asthma[40] and, in children sensitized to a single allergen, reduced risk of the subsequent development of sensitization to further allergens[41]. Sublingual allergen immunotherapy (SLIT) offers an alternative route of allergen administration that reduces the risk of potentially serious allergic side effects when compared with subcutaneous SIT[42]. At present, the relative efficacy of SLIT compared with SIT is not known, although the long-term benefit of both has been claimed[43,44]. The mechanisms through which SIT and SLIT produce their beneficial clinical effects are becoming clearer[45]. SIT reduces clinical symptoms by inhibiting allergen-specific T_H2 cells in favour of a T_H1 response (immune deviation)[46] and inducing regulatory lymphocytes carrying the CD4 and CD25 antigens and CD4+ CD25- T_H3 (immune tolerance)[47–49] (FIG. 6). Increased numbers of macrophages expressing interleukin (IL)-12 have also been found in skin biopsies at the site of SIT[50], which provides a stimulus for the development of T_H1 rather than T_H2 lymphocytes[51]. The failure of T_H1 cells to reverse an established T_H2 response has focused attention on T_H3 cells, which actively suppress inflammation by releasing IL-10 and transforming growth factor-β and regulatory lymphocytes carrying the CD4+ CD25+ antigens[6], which are selectively regulated by the transcription factor forkhead box P3 (REF. 52). Possible ways of increasing the number or activity of regulatory T cells include manipulation of the glucocorticoid-induced tumour-necrosis factor receptor (GITR)[53] or selectively activating CCL4 chemokine receptors[54]. Although offering potential, all of these approaches are still experimental. Specific immunotherapy also increases allergen-specific 'blocking' IgG1 and IgG4 antibodies, a variable decline in allergen-specific IgE, and reduces both the number and activation state of mucosal mast cells,

SIT
Allergen-specific immunotherapy that uses incremental small doses of subcutaneously injected allergen to induce immunological tolerance.

SLIT
Sublingual immunotherapy, which uses a higher concentration of allergen than used in SIT to induce tolerance via the buccal mucosa and draining lymphoid tissue.

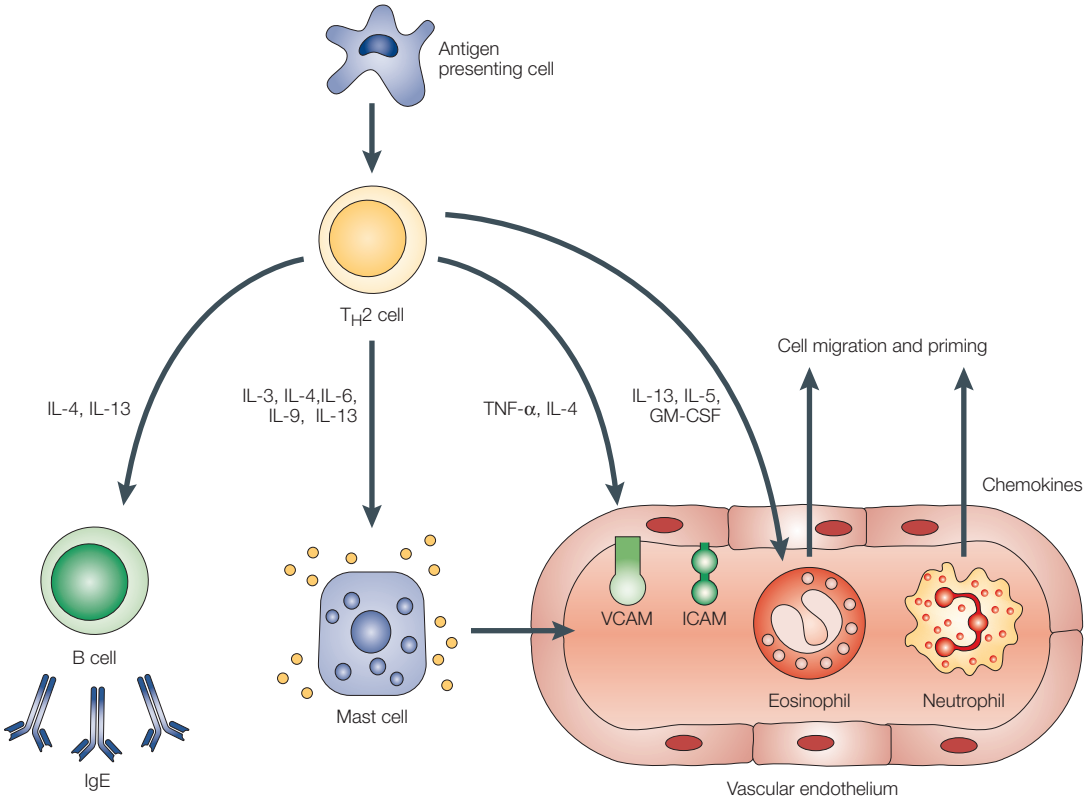

Figure 5 | **Interactions between cytokines and endothelial cells in the recruitment of inflammatory leukocytes.** Cytokines such as tumour-necrosis factor-α (TNF-α), interleukin (IL)-1 and IL-4 released in the nasal mucosa upregulate adhesion molecules on vascular endothelium, which stimulates the adhesion of circulating leukocytes to mucosal blood vessels. The further expression of chemokines directs the recruitment of circulating leukocytes, in particular eosinophils and basophils, into the nasal mucosa. GM-CSF, granulocyte–macrophage colony-stimulating factor; ICAM, intercellular adhesion molecule; IgE, immunoglobulin E; VCAM, vascular cell adhesion molecule.

basophils and eosinophils[55]. Even though SIT is efficacious, its administration can be associated with local and systemic allergic reactions, and so a variety of strategies to reduce this and to enhance efficacy are being investigated. These include the use of allergen peptide-based immunotherapy, allergen protein immunotherapy combined with anti-IgE, and allergen protein conjugated to CpG DNA.

Peptide-based immunotherapy

The therapeutic potential of allergen peptide immunotherapy has been demonstrated in mouse models of allergic inflammation[56]. The rationale for using short peptides is to reduce the potential for allergic side effects while retaining the beneficial effect of peptide epitopes recognized by T cells in modifying their response to allergens, because peptides are unable to crosslink $Fc_{\varepsilon}R1$-bound IgE on mast cells and basophils. As the local and systemic side effects of protein-based SIT are largely caused by IgE-mediated mast-cell activation, the safety and efficacy of peptides has been taken advantage of in the treatment of cat allergy using several overlapping peptides derived from chain 1 or 2 of the major cat allergen *Fel d1*[57]. Weekly subcutaneous immunization with 27-amino-acid peptides derived from *Fel d1* led to a reduction in rhinitis symptom scores on exposure to cats in an exposure room. However, one hour or more after the first peptide dose, allergic side effects occurred in 16 out of 24 patients. A single systemic administration of peptides of 16 or 17 amino acids in length derived from the cat allergen *Fel d1* to patients with asthma induces allergen-specific hyporesponsiveness, both to peptide re-challenge and to cutaneous challenge with whole-protein allergen[58]. However, with the initial administration peptide therapy can also induce adverse effects, such as isolated late asthmatic reactions, which are MHC-restricted and T-cell mediated[59], but which are not associated with elevated airway levels of histamine or eosinophils[60]. Clinical trials of the shorter *Fel d1* peptides are in progress.

DNA-based immunotherapy

CpG DNA oligonucleotides. Immunostimulatory DNA sequences containing CpG motifs are strong inducers of a T_H1 immune response to antigen and have therefore been investigated in the treatment of T_H2-mediated diseases such as allergic rhinitis and asthma[61,62]. Immunostimulatory DNA sequences contain unmethylated CpG dinucleotides within a hexamer that follows the formula 5′-purine–purine–CG–pyrimidine–pyrimidine–3′ (for example, 5′-GACGTC-3′) or 5′-purine–TCG–pyrimidine–pyrimidine-3′ (for example,

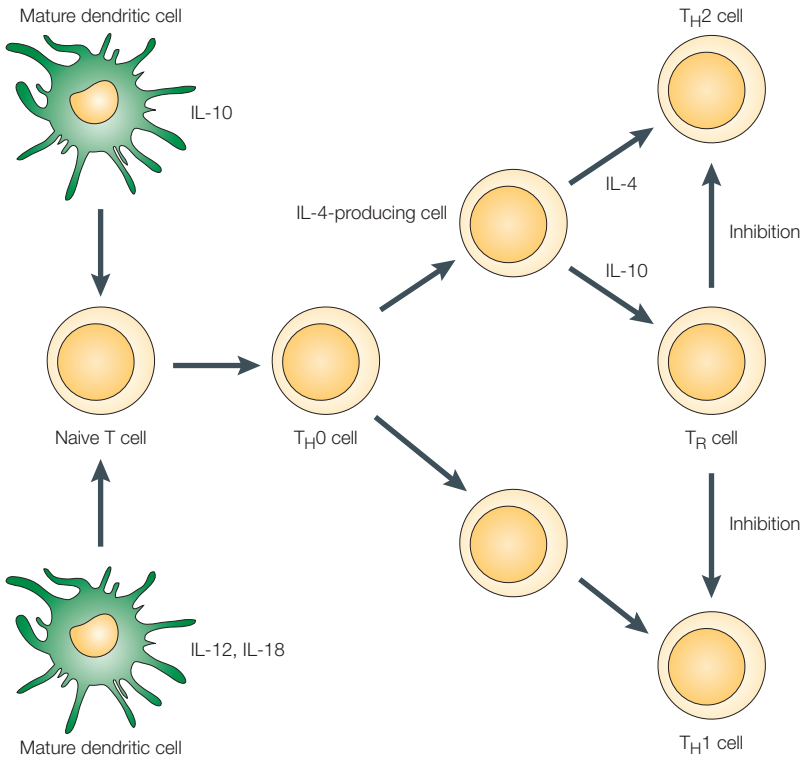

Figure 6 | **Cytokine reactions involved in shaping the immune response to inhaled allergens.** Interleukin (IL)-12 and IL-18 deviate the naive T-cell response towards a T_H1 phenotype whereas low IL-12 or IL-18 and high IL-10 polarizes the response in favour of T_H2 cells which are then maintained by IL-4. In the presence of further IL-10, a sub-population of CD4+ CD25+ T regulatory cells develop with the capacity to inhibit both T_H1 and T_H2 responses. (Modified with permission from REF. 6 © Macmillan Magazines Ltd.

5′-GTCGTC-3′) (FIG. 7). CpG DNA inhibits T_H2 responses to antigen indirectly by influencing the function of cells of the innate immune system, rather than exerting direct effects on T lymphocytes. Studies with TLR9-deficient mice have demonstrated that these receptors of the innate immune response are essential in mediating the immunostimulatory activity of CpG DNA[63], which is characterized by the production of IL-12, IL-18, interferon-α (IFN-α), IL-6 and IL-10 (REF. 64). The cytokine environment induced by CpG DNA is highly effective at reducing the levels of expression of T_H2 cytokine receptors (for example, the IL-4 receptor)[65]. In mouse models of allergic lower-airway inflammation, CpG DNA prevents as well as reverses T_H2 cytokine expression, eosinophilic inflammation and epithelial mucus production[66–68]. In a mouse model of allergic rhinitis, CpG DNA administration prevented both the development of nasal symptoms and eosinophilic inflammation[69]. At present there are no results of human studies with CpG DNA in allergic rhinitis, although studies are in progress, the outcome of which are eagerly anticipated.

CpG DNA–allergen protein conjugate. The use of an allergen protein conjugated to CpG DNA for the treatment of allergic diseases has certain theoretical advantages compared with unconjugated CpG DNA. The rationale behind this is that by physically linking CpG DNA to protein allergens the likelihood of their co-delivery to the same APC increases, thereby resulting in an amplified, allergen-specific T_H1 response[70] that is much less likely to occur when CpG DNA and allergen are administered separately. The APC would process and present the antigen to T_H cells, while CpG DNA would induce the same APC to release IL-12, thereby biasing the naive T_H0 cell to differentiate toward a T_H1 and possibly T_H3 and regulatory lymphocyte phenotypes. The ability of CpG DNA conjugated to a protein allergen to modify an allergic response has best been studied with CpG DNA conjugated to either *AMB A* 1 (FIG. 7), the major short ragweed allergen, or to ovalbumin protein allergen[71,72]. In mice, injection of a CpG DNA–*amb a* 1 conjugate is much more effective than the injection of a mixture of *amb a* 1 and CpG DNA in inducing a T_H1 immune response, as well as in reversing a pre-existing T_H2-biased immune response to *amb a* 1. CpG DNA–*amb a* 1 protein conjugate inhibits the release of histamine from basophils from ragweed-induced allergic rhinitis *in vitro*, and significantly reduces the size of the immediate hypersensitivity skin-test response to *amb a* 1 allergen *in vivo*[73] in the same patients. Preliminary results of Phase I and II studies in humans with allergic rhinitis indicate that the CpG DNA–*amb a* 1 protein conjugate reduces allergic rhinitis symptom scores during the ragweed season in subjects immunized before the ragweed season with the CpG DNA–*amb a* 1 protein conjugate[74]. Full-scale clinical trials have now started.

Targeting IgE

IgE is the principal triggering mechanism for allergic rhinitis. IgE interacts with both $FC_\varepsilon R1$ and the lower-affinity receptor $FC_\varepsilon R2$ (CD23). Differentiation of B cells into IgE-secreting plasma cells requires at least two distinct signals in IL-4 (or IL-13) and CD40L on the surface of T_H2 cells with CD40, a co-stimulatory molecule on B cells which triggers ISOTYPE SWITCHING to IgE (FIG. 4). IgE binds to the α-chain of the tetrameric $Fc_\varepsilon R1$ complex on mast cells, basophils, monocytes and dendritic cells. The molecular interactions responsible for high-affinity binding are complex and involve several sites in the $C_\varepsilon 3$ domain of IgE[75]. A murine monoclonal antibody, MAE11 (Genentech), has been generated that recognized the same residues in the Cr3 domain that are responsible for the binding of $Fc_\varepsilon R1$. To avoid sensitization with foreign proteins, a humanized monoclonal antibody containing 95% human IgG1 and ~5% murine IgE-binding epitope has been constructed (omalizumab (Xolair; Novartis))[76]. This antibody recognizes IgE selectively, inhibits binding of IgE to both $Fc_\varepsilon R1$ and $Fc_\varepsilon R2$, but does not bind to IgE already attached to $Fc_\varepsilon R1$ and, therefore, fails to initiate mast-cell or basophil activation (FIG. 8). When administered as two-weekly or one-monthly subcutaneous injections, omalizumab decreases circulating free IgE by >90% by forming small (~1000 kDa), non-complement-fixing complexes that are eliminated by the reticuloendothelial system without causing side effects. Omalizumab is

AMB A 1
The major allergen of ragweed pollen.

$FC_\varepsilon R1$ AND $FC_\varepsilon R2$
The high- and low-affinity receptors for IgE present on mast cells, basophils and dendritic cells.

ISOTYPE SWITCHING
The capacity of B lymphocytes to redirect their immunoglobulin (Ig) synthesis from IgM to another Ig subclass

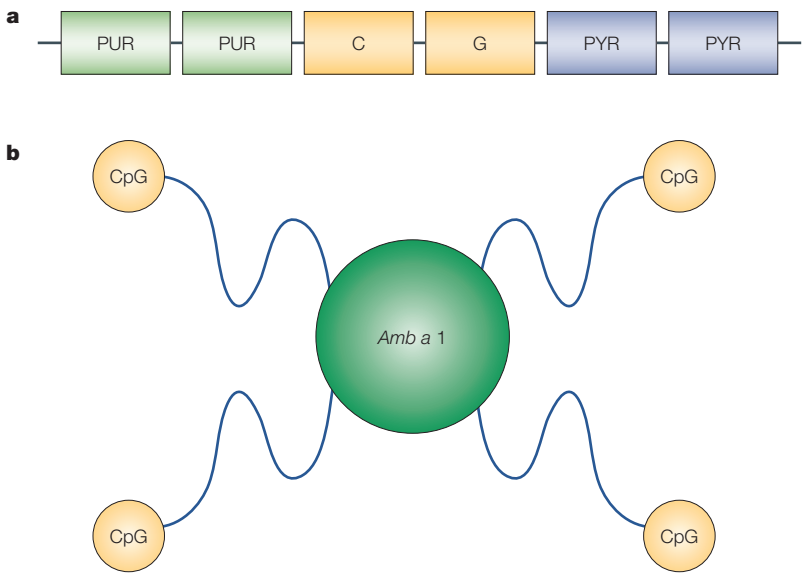

Figure 7 | **Generic structure of CpG DNA oligonucleotides and a conjugate. a** | Generic structure of CpG DNA oligonucleotides. **b** | CpG DNA conjugated to the ragweed pollen allergen *amb a* 1. PUR, purine; PYR, pyrimidine.

efficacious in the treatment of moderate–severe asthma, an indication for which it has received approval for use in the United States[77], and has also shown efficacy in two clinical trials of seasonal allergic rhinitis[78,79]. However, in the rhinitis trials the anti-IgE therapy was probably started too late and/or given in too low a dose to totally prevent the rise in pollen-specific IgE during the season, and as a result efficacy was not as high as might have been predicted from a knowledge of the sentinel role of IgE in this disease. In children with allergic rhinitis, a combination of SIT with anti-IgE for 24 weeks was more efficacious than when either treatment was given alone[80]. This provides a good case for using anti-IgE to protect against anaphylactic responses during SIT. Both systemically[80] and in the airways (R. Djukanovic, personal communication), omalizumab therapy is accompanied by a marked reduction in inflammatory leukocytes and expression of $Fc_{\varepsilon}R1$, which, if not occupied by IgE, becomes internalized[81]. Loss of functional $Fc_{\varepsilon}R1$ receptors on dendritic cells, as well as mast cells and basophils, contributes to the efficacy of anti-IgE therapy in allergic disease[82]. Related approaches include vaccines comprising the peptide epitopes responsible for IgE-binding to its receptors[83,84], and antibodies that target the IgE-binding site on the α-chain of $Fc_{\varepsilon}R1$ (REF. 85). A monoclonal blocking antibody to soluble CD23 (IDEC-152; IDEC Pharmaceuticals) has recently been shown to reduce circulating total IgE levels by blocking the effects of the soluble form of this receptor that augments IgE production by B cells (L. Rosenwasser, personal communication). However, a recent clinical trial in rhinitis has failed to reveal efficacy despite a 50% reduction in total serum IgE (T. B. Casale, personal communication). This observation emphasizes the importance of reducing circulating IgE by >90% for this type of anti-allergic strategy to be successful in treating the disease. Low-molecular-weight molecules that interfere with IgE–$Fc_{\varepsilon}R1$ binding are also under development.

CAMS
Cell adhesion molecules including intercellular (ICAM) and vascular (VCAM) members that are expressed on endothelial cells, upregulated by cytokines and involved in leukocyte adhesion and activation.

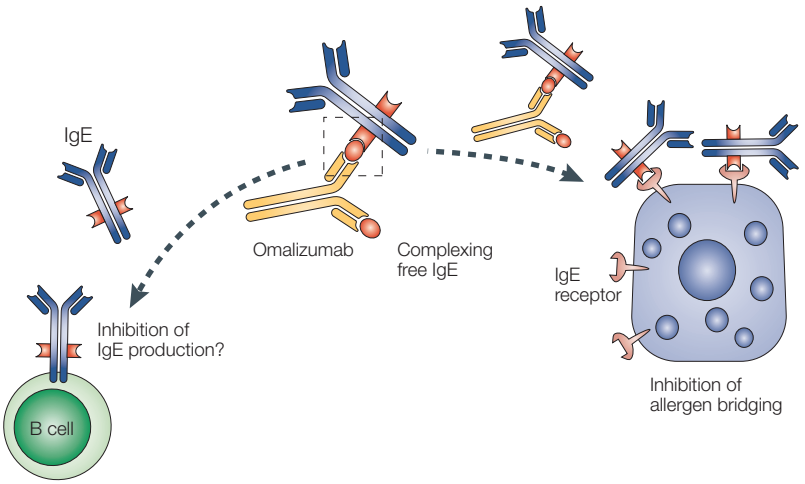

Figure 8 | **Interaction of the non-anaphylactic IgG anti-human monoclonal antibody omalizumab with IgE to prevent mast cell sensitization.** Omalizumab binds with high affinity to an epitope in the CR3 region of immunoglobulin E (IgE) to prevent its interaction with $Fc_{\varepsilon}R1$ and $Fc_{\varepsilon}R2$ on effector cells.

Targeting cytokines and chemokines

One of the difficulties in deciding on which cytokines or chemokines to target in the treatment of allergic rhinitis is the large variety of them that are expressed at sites of allergic inflammation, as well as their overlapping functions. However, studies in rheumatoid arthritis have demonstrated that the targeting of a single cytokine (in these cases, tumour-necrosis factor-α (TNF-α)) can have a major effect on a complex disease, providing that the cytokine acts sufficiently early in the inflammatory cascade. In allergic inflammation, research has focused particularly on individual T_H2 cytokines (for example, IL-4, IL-5, IL-9 and IL-13) and chemokines that attract cells to sites of allergic inflammation[8,86]. Preclinical studies in mouse models of allergic inflammation have validated the therapeutic potential of individually targeting several T_H2 cytokines[87,88], but at present there are only limited studies of cytokine antagonists in humans with allergic inflammation.

Although there are no published studies of cytokine antagonists in humans with allergic rhinitis, studies of blocking antibodies and soluble receptors in asthma have provided some insight into the utility of this approach for rhinitis, because it shares some of the features of asthma in responding to inhaled allergens. IL-4 mediates important pro-inflammatory functions in allergic inflammation, including induction of the IgE isotype switch (FIG. 4), expression of vascular CELL ADHESION MOLECULE-1 (VCAM-1), promotion of eosinophil transmigration across the endothelium (FIG. 5), stimulation of mucus production and promotion of T_H2 lymphocyte differentiation, leading to further release of IL-4, IL-5, IL-9 and IL-13 (REF. 87). The therapeutic potential of a recombinant soluble IL-4 receptor (IL-4R; altrakincept (Nuvance; Immunex Corp.)) as an IL-4 antagonist has been studied in asthmatics. In two small studies,

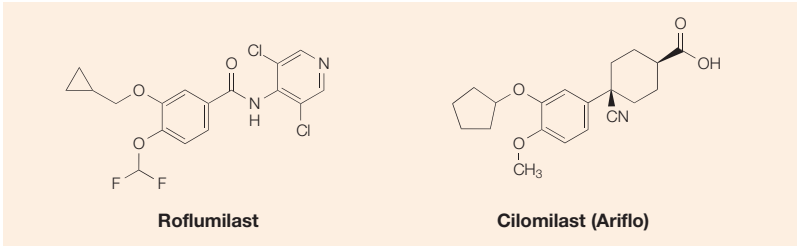

Figure 9 | **Chemical structure of two selective phosphodiesterase type IV inhibitors.** Rofumilast (left) and Cilomilast (right) as examples of type IV phosphodiesterase inhibitors in clincial trials for allergic airways disease.

treatment with Nuvance improved asthma symptom scores and pulmonary function, reduced β_2-agonist rescue use, as well as lowering levels of exhaled nitric oxide[89,90]. However, two large Phase III studies in moderate–severe asthma disappointingly failed to reveal efficacy, possibly due to dose limitations and the short duration of action of Nuvance. IL-4R can also be blocked with mutant forms of IL-4 which themselves exert little or no agonist activity. A naturally occurring alternative splice variant of human IL-4 (IL-4δ2) is expressed at high levels in airway cells and competes with IL-4 to inhibit its effects[91]. A mutant form of IL-4 in which tyrosine at position 124 is replaced by aspartic acid (IL-4 Y124D) binds with high affinity to the IL-4Rα but is without biological effect on either T cells or B cells[92]. A human recombinant IL-4 double mutant serving as a neutral antagonist has been developed which has given promising results in primate models of asthma, but is limited by its relatively short duration of action (P. Harris, personal communication).

IL-13 shares many similar biological effects with IL-4 — including upregulation of endothelial adhesion molecules, epithelial mucous metaplasia and eosinophilic inflammation — but has no effects on T cells, because they do not usually express IL-13 receptors[93,94]. Targeting IL-13 has been investigated in allergic inflammation predominantly in mouse models of asthma, in which its antagonism inhibits the allergic inflammatory response in the lower airways[95]. Allergen challenge increases the level of expression of IL-13 in the nasal mucosa *in vivo*[96], whereas *in vitro* IL-13 increases the number of secretory cells in human nasal epithelial cells[94]. Both IL-4 and IL-13 signal through a common receptor, IL-4Rα[97]. Although IL-13Rα1 forms a high-affinity receptor-signalling complex when co-expressed with IL-4Rα, a second IL-13 receptor, IL-13Rα2, binds IL-13 with higher affinity and, in contrast to IL-13Rα1, is also found as a soluble receptor in serum. The cytoplasmic region of murine IL-13Rα2 does not possess an obvious signalling motif or JAK/STAT (signal transducers and activators of transcription)-binding sequence, indicating that it is a dominant negative inhibitor or decoy receptor, as confirmed in studies of mice with targeted deletion of IL-13Rα_2 (REF. 98). In common with soluble IL-4R, soluble IL-13 receptors could represent a novel therapeutic strategy to inhibit IL-13-mediated inflammation. Specific blockade of IL-13 by administration of the soluble IL-13α2 chain, which binds only IL-13 with high affinity[97,99], but not IL-4, reverses airway hypersensitivity and mucous metaplasia in allergen-challenged mice[100]. In mice (and possibly in humans), IL-13 has been implicated in airway remodelling and disease chronicity through its effects on epithelial cells and fibroblasts, which it converts into goblet cells and myofibroblasts, respectively[99]. Both monoclonal blocking antibodies against IL-13 and soluble human recombinant IL-13Rα2 are in clinical trials. An IL-13 double mutein has also been synthesized that blocks IL-13 effects in human cells *in vitro*[101]. At present there are no results of studies targeting IL-13 in human allergic rhinitis.

Human studies with anti-IL-5-blocking monoclonal antibodies in asthma have demonstrated dramatic effects in reducing sputum and blood eosinophilia, but interestingly not on the late-phase lower airway response to inhaled allergen, nor on measures of clinical asthma, thereby raising questions about the previously held notion of the pivotal roles of IL-5 and eosinophils in allergic asthma[102,103]. Subsequent studies have demonstrated that anti-IL-5 is less effective at inhibiting eosinophils in the lower airways (~50%) than in the blood (~90%) and sputum (~75%), indicating that partial depletion of tissue eosinophils might be insufficient for clinical efficacy in a complex disease such as asthma[104]. Studies using IL-5-deficient mice in a model of allergic rhinitis also indicate that reductions in IL-5 and eosinophils might not completely suppress clinical symptoms or nasal histamine hyperresponsiveness[105]. Monoclonal anti-IL-4 antibodies inhibit IgE production in mice[106,107], but this does not influence airway eosinophilia or lower-airway hyperresponsiveness (AHR); by contrast, anti-IL-5 antibodies inhibit airway eosinophilia but not IgE production or AHR. However, the combined anti-IL-4 and anti-IL-5 antibody inhibited all three responses of the allergic airway response[107].

The C-C chemokines, including chemokine ligand 11 (also known as eotaxin), RANTES (regulated on activation, normal T-cell expressed and secreted), monocyte chemoattractant protein (MCP)-3 and MCP-1, are particularly relevant to allergic inflammation, as increased levels of these chemokines are detected in the nasal mucosa following allergen challenge and all interact with the CCR3 receptor on eosinophils, basophils and mast cells[108]. Activation of CCR3 receptors by application of eotaxin to the nasal mucosa induces an influx of eosinophils[109]. Studies demonstrating that an 11-amino-acid synthetic peptide inhibits nasal influx of neutrophils and protein exudation induced by nasal challenge with IL-8 in normal subjects[110] indicate the potential for inhibiting the function of chemokines in the nasal mucosa with a topically applied therapy. Human studies in asthma and rhinitis are in progress using both blocking antibodies to eotaxin (T. Taylor-Clark, personal communication) or small-molecule CCR3 antagonists. The combination of anti-IL-5 antibody with a chemokine antagonist active on eosinophils (for ex ample, anti-CCR3) might be a more effective approach to the inhibition of eosinophilic tissue inflammation than the use of either therapy alone. Support for this

Table 1 | **Current development of selected therapeutics for allergic rhinitis**

Therapeutic	Preclinical	Phase 1	Phase 2	Phase 3	Registered
H_1 antihistamines					+
H_4 antihistamines	+				
$CystLT_1$ antagonists					+
$CystLT_2$ antagonists	+				
PGD_2 DP_1 antagonist		+	+		
PDG_2 DP_2 antagonist	+				
Tryptase inibitors		+			
Mast cell ion channel blockers	+				
Mast cell signalling inhibitors		+			
Neuropeptide antagonists		+	+		
SLIT				+	
Peptide-based IT		+			
CpG ISS	+	+			
CpG-allergen conjugate			+		
Anti-human IgE monoclonal antibodies					
IgE peptides	+				
IL-4 antagonists/monoclonal antibodies		+	(+) soluble receptor		
IL-13 antagonists/monoclonal antibodies		+			
Eotaxin monoclonal antibody		+			
CCR3 antagonists		+			
Mycobacterial vaccines		+			
Probiotics			+		
VLA-4 antagonsits monoclonal antibodies			+		
P-selectin antagonists		+			
E-selectin/ICAM-1 antagonists		+			
Selective PDE4 inhibitors			+		

ICAM, intercellular adhesion molecule; IgE, immunoglobulin E; IL, interleukin; ISS, immunostimulatory sequence; SLIT, sublingual allergen immunotherapy; PDE, phosphodiesterase; PDG, prostaglandin; VLA, very late antigen.

notion comes from studies in mouse models in which lower-airway eosinophilic inflammation is ablated in mice deficient in both of the genes encoding IL-5 and eotaxin when compared with the single-gene knock-out strain[111].

Enhancing T_H1 responses

Another approach to regulate aberrant T_H2 immune responses in allergic disease is to administer T_H1-polarizing cytokines, such as IFN-γ, IL-12 or IL-18. Although animal studies indicate that such interventions can prevent the development of allergic responses, clinical studies in which this approach has been used in an attempt to reverse established allergic responses have been disappointing[112–114]. Moreover, concern has also been expressed over whether enhancing T_H1 immunoreactivity might lead to enhancement of the inflammatory response, as has been shown in mice[115]. Slightly more promising has been the use of mycobacterial vaccines, especially *MYCOBACTERIUM VACCAE*, which is highly effective in preventing allergic sensitization in mice[116], has some effect in reducing established airway allergic responses in humans[117] and might operate via regulatory T cells[118]. There remains controversy over whether vaccines prepared from mycobacteria are able to redirect the immune response away from an allergic phenotype during early life, although clinical trials to establish this are ongoing. Programming the mucosal immune response by ingested bacteria (probiotics) has also produced some encouraging results showing that perinatal administration of *Lactobacillus* spp. reduced the development of eczema by 50% during the first two years of life[119]. However, a recent trial on birch-pollen allergy failed to show any effect of probiotic therapy on established disease[120]. Vaccines aimed at deliberately enhancing T_H1 responsiveness during the first three to five years of life so as to prevent the onset of atopy in at-risk children hold most promise, although such an approach brings with it concern about potential increased risks of diseases associated with enhanced T_H responses, such as diabetes mellitus and inflammatory arthritis.

Targeting adhesion molecules

As the recruitment of circulating leukocytes (that is, mast cell precursors, eosinophils, basophils and T lymphocytes) from the circulation into the nasal mucosa is a prominent feature of allergic rhinitis, the targeting of

MYCOBACTERIUM VACCAE
A non-disease-causing mycobacterium found in the soil as a saprophyte.

adhesion molecules on the leukocyte or endothelial cell surface has been investigated as an approach to inhibit allergic inflammation. Following nasal allergen challenge, cytokines and mediators are released which upregulate the expression of endothelial cell adhesion molecules in blood vessels in the nasal mucosa[121]. The importance of endothelial P-SELECTIN, which is highly expressed in the nasal mucosa[122], to eosinophil recruitment is indicated by studies of mouse models of allergic inflammation. These showed that there is significant inhibition of the initial tethering of eosinophils to endothelium, as well as inhibition of eosinophilic tissue recruitment, in P-selectin-deficient mice[123]. Inhibition of P-selectin is likely to reduce eosinophilic inflammation to a greater degree than targeting E-selectin, as E-selectin is more effective at inhibiting neutrophil, rather than eosinophil, recruitment[124]. Subsequent to endothelial tethering, eosinophils firmly adhere to either ICAM-1 or VCAM-1. Blockade of these receptors in mouse models of allergic inflammation, and inhibition of eosinophilic tissue recruitment in ICAM-1-deficient mice, results in marked inhibition of firm adhesion of eosinophils to endothelium[123]. As inhibition of either P-selectin or ICAM would also inhibit neutrophil tissue recruitment, and potentially predispose to infection, research has focused on the VCAM-1 adhesion pathway that mediates eosinophil but not neutrophil recruitment. Eosinophils, basophils, monocytes and T cells, but not neutrophils, express high levels of VERY LATE ANTIGEN-4 (VLA-4), the ligand for VCAM-1 (REF. 125). Binding of VLA-4 to the CS-1 region of fibronectin also induces eosinophil activation[126], such that by targeting VLA-4, cell activation as well as cell recruitment might be inhibited. In animal models of asthma, inhibiting VLA-4 reduces AHR without significantly inhibiting eosinophil tissue recruitment in some[127] but not all models[128]. At present there are no published studies of anti-adhesion therapy in allergic rhinitis, although two Phase III trials in asthma of two different inhaled VLA-4 antagonists have failed to reveal efficacy. It remains possible that VLA-4 antagonists need to be administered systemically to prevent this integrin from functioning on microvessels. Of relevance to this idea, a placebo-controlled trial with an anti-VLA-4 antibody in patients with relapsing multiple sclerosis demonstrated that this treatment led to few inflammatory brain lesions and fewer relapses during a six-month period[128].

SELECTINS
Glycoproteins that are expressed on endothelial cells involved in leukocyte rolling (P selectin) or adhesion (E-selectin).

VERY LATE ANTIGEN-4
(VLA-4). An integrin α4β1 that selectively binds to VCAM-1 and to CS-1 region of fibronectin.

Selective phosphodiesterase inhibitors

One promising development is the use of selective phosphodiesterase 4 (PDE4) inhibitors (FIG. 9), which exert anti-inflammatory activity by blocking the hydrolysis of cyclic 3′5′-AMP in lymphocytes, eosinophils, neutrophils and monocytes, thereby attenuating their release of mediators and cytokines, including IL-4, IL-5, IL-10 and granulocyte–macrophage colony-stimulating factor[129]. Although known to be effective in the treatment of asthma and chronic obstructive pulmonary disease, oral once-daily therapy with the PDE4 inhibitor roflumilast[130] in patients with allergic rhinitis subjected to repeated allergen exposure proved to be efficacious, especially on nasal blockage[131], warranting further, larger comparative studies.

Conclusion

The selective inhibition of mast cells, new methods to induce allergen-specific immunological tolerance, blockade of IgE and strategies to reduce secondary leukocyte recruitment and activation seem to be the most promising therapeutic targets for allergic rhinitis (TABLE 1). However, although a more detailed knowledge of the cellular and molecular mechanisms of the immunological and inflammatory pathways involved in disease pathophysiology will undoubtedly lead to the development of new therapeutics, the real challenge in this disease will be to understand the reasons for the continued upward trends in its incidence and whether the mechanisms underlying this could provide new clues for the primary prevention of allergy.

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