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Therapies for allergic inflammation: refining strategies to induce tolerance

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Current therapies for asthma and allergy are relatively safe and effective at controlling symptoms but do not change the chronic course of disease. There is no established method to prevent asthma and allergy, and major unmet needs in this area include the better control of the severe forms of these diseases and the developments of curative therapies. Two major therapeutic strategies for asthma and allergy are currently being developed, and I here discuss the advances and challenges for future therapeutic development in these two areas. The first approach, allergen-specific immunotherapy, aims to induce specific immune tolerance and has a long-term disease-modifying effect. The second approach is the use of biological immune response modifiers to decrease pathological immune responses. Combination strategies using both of these approaches may also provide a route for addressing the unmet clinical needs in allergic diseases.

Allergic inflammation is caused by the development of an allergen-induced immune response and can lead to several diseases, including asthma, allergic rhinoconjunctivitis, anaphylaxis, urticaria and atopic dermatitis[1]. It is now becoming clear that allergic diseases are complex disorders and that there are several disease variants caused by different underlying cellular and molecular mechanisms. A limited knowledge of the pathophysiology of these disease subgroups has been the greatest obstacle to identifying consistent correlations between genes, the environment and the different disease subgroups. Although there are several clinically relevant phenotypes for asthma, atopic dermatitis and urticaria, these phenotypes might not provide any insights into the mechanisms that underpin the diseases. It is now thought that some clinical trials may have previously been unsuccessful because they were performed without attempting to classify patients into subgroups defined by distinct pathophysiologies, namely 'endotypes'[2]. In asthma, phenotypes describe clinically relevant characteristics but do not necessarily give insights into the underlying pathological mechanisms of the disease. In contrast, asthma endotypes describe disease subtypes on the basis of cellular and molecular pathogenic mechanisms[3]. In addition, the results of large-scale genetic studies, particularly studies of asthma and atopic dermatitis, may have been inconclusive because of the bulk patient selection approaches used in many of these studies (**Box 1**).

Despite substantial improvements in treatments for asthma and allergy, approximately 5–10% of all people with these disorders have inadequately controlled severe persistent asthma or severe atopic dermatitis. These individuals use a large proportion of public health resources devoted to the treatment of asthma and allergy, and new, more effective therapies are urgently required. The possibility of curing allergic diseases is an essential issue for research because the medications currently used to treat these diseases, such as antihistamines, leukotriene receptor antagonists and glucocorticoids, only temporarily relieve symptoms by suppressing inflammation[4]. However, a long-term cure for allergic diseases can be achieved through the use of allergen-specific immunotherapy (allergen SIT), which has a disease-modifying effect and might also lead to decreased requirements for anti-inflammatory and symptomatic medications[5,6]. The basic principle of allergen SIT is to induce immune tolerance to allergens through multiple cellular and molecular mechanisms by administering repeated, increased doses of the causative allergen. Almost all of the immune tolerance mechanisms involved in responses to SIT are currently being targeted by drug discovery programs.

Unmet needs in treatment and advances in molecular biology and immunology have also spurred the development of new biological immune response modifiers to treat allergy and asthma. Such biological modifiers (biologicals) include therapeutic antibodies, soluble receptors, cytokines and small molecules, as well as combinations of these agents that can target effector molecules at various points in the immune and inflammatory pathways on different immune cells. So far, over 30 monoclonal antibodies have been approved for various clinical indications, particularly for autoimmune disorders, organ transplantation, infectious diseases and cancer. More than 300 biologicals are currently in clinical trials, and some researchers have suggested that there will be a switch from the use of chemicals to the use of biologicals in drug development within the next 10 years. Although this review will focus on the results from human clinical trials of such biological therapeutics, I also highlight promising therapeutic candidates from preclinical

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BOX 1 Problems in drug development for the treatment of asthma

- Complexity of the whole disease spectrum
- Complexity of molecular mechanisms
- Limited biomarkers for subgrouping and endotyping
- Limited information on how to improve on existing therapies (for example, currently used inhaled steroid and β-adrenergic agonist combination therapy is effective and relatively inexpensive)
- Low patient adherence, which is a general problem for chronic diseases
- Small animal models are poorly predictive. Most drugs that are effective in mouse models have failed in clinical trials
- Individual outcomes are different, and different outcomes cannot be distinguished using a bulk approach
- It is not possible to study the combination of two new biologicals that may potentiate each other until one of them is approved; however, biologicals are unlikely to be effective when used alone

and mouse studies, which will need to be validated and tested in humans to see whether they fulfill their initial promise.

As there is evidence that allergen SIT might offer the possibility of a cure for asthma and allergic diseases, many research efforts are now concentrating on combining allergen SIT with new biological immune modifiers (**Table 1**). Such combination strategies could be advantageous over individual therapies in reducing side effects, providing synergistic effects that may allow for the treatment of persistent disease and providing more flexibility for treatments to be tailored according to different disease endotypes. Devising the separate treatment arms of the combination of allergen SIT and a biological immune modifier will pose substantial challenges, and we still have a long way to go before we can bring such combinations to the clinic.

Mechanistic insights into immune tolerance to allergens

The concept of inducing immune tolerance has become crucial in the development of prevention and treatment strategies for many diseases in which dysregulation of the immune system has a key role, such as allergy, asthma, autoimmunity, organ transplantation and infertility. Immune tolerance to allergens is characterized by the establishment of long-term clinical tolerance[5,6]. In addition to the immune responses induced by various modes of allergen SIT, the development of healthy immune responses in beekeepers and cat owners who are exposed to high doses of allergens has been intensively studied to understand the mechanisms of allergen tolerance in humans[7,8]. Although several details of the tolerance process have yet not been elucidated, mechanisms involved include changes in the profiles of allergen-specific memory T and B cell responses and the production of specific antibody isotypes to skew the immune response in a noninflammatory direction, as well as decreased activation, tissue migration and degranulation of mast cells, basophils and eosinophils (**Box 2**).

Rapid desensitization of mast cells and basophils by allergens. Several mechanisms have been proposed to operate to ensure that mast cells and basophils are unresponsive to environmental proteins, even in the presence of specific immunoglobulin E (IgE). Notably, in patients, even after the first injection of allergen SIT, very early decreases in the susceptibility of mast cells and basophils to degranulation and decreases in systemic anaphylaxis are observed, although all of the treated individuals have high quantities of specific IgE. Although the underlying molecular pathways of this response are not yet elucidated, this mechanism seems similar to the one observed when these two immune-cell types are rapidly desensitized in anaphylactic reactions to drugs[9]. Anaphylaxis is associated with the release of inflammatory mediators from both mast cells and basophils, and successful hyposensitization alters the magnitude of the mediator release[10]. The release of these inflammatory mediators in low quantities (below the required dose for systemic anaphylaxis) may affect the threshold of activation of the mast cells and basophils[10,11].

Regulatory T (T_{reg}) cells and peripheral T cell tolerance to allergens. The induction of peripheral T cell tolerance, which is characterized primarily by the generation of allergen-specific T_{reg} cells, is an essential step in allergen SIT[12,13] (**Fig. 1**). Peripheral tolerance is initiated by the cytokines interleukin-10 (IL-10) and transforming growth factor-β (TGF-β), which are produced in increasing amounts by the allergen-specific T_{reg} cells as allergen SIT proceeds[12,13]. Roles have been shown for both IL-10–secreting type 1 regulatory T ($T_{reg}1$) cells or forkhead box P3 (FOXP3)-positive T_{reg} cell subsets, suggesting that there is an overlap in the functions and characteristics of the cytokines and surface molecules of these inducible subsets of T_{reg} cells in humans[14]. $CD4^+CD25^+$ T_{reg} cells from atopic donors showed a reduced potential to inhibit $CD4^+CD25^-$ T cell proliferation, which is an indicator of peripheral allergen tolerance[15]. Allergen SIT has been shown to increase the number of $FOXP3^+CD25^+CD3^+$ cells in the nasal mucosa, and this increase associates with clinical efficacy and the suppression of seasonal allergic inflammation, supporting a putative role for T_{reg} cells in the induction of allergen-specific

Table 1 Features of and challenges for allergen SIT vaccines and biological immune response modifiers

Allergen SIT vaccine	Biological immune response modifier
Should induce long term allergen tolerance (curative)	Should decrease or suppress the pathological immune response
Should achieve clinical success in a short time with few doses	Persistent improvement should be achieved when the treatment is stopped
Should target individuals with an allergy to identified allergens	Should target inflammation that is refractory to conventional therapies
Biological and/or immunological markers should be identified for patient selection and assessment of which population should be targeted, when to start and stop therapy and how to follow the patients	Should be developed together with its specific biomarkers that give information on which population should be targeted, when to start and stop therapy and how to follow the patients
Use of multiple allergens at the same time is possible	Combined use of two or more biologicals is currently not possible
Molecular and cellular mechanisms of allergen or antigen tolerance have been mostly elucidated	There is a substantial amount of missing knowledge in the physiopathology of tissue inflammation
Was initiated 100 years ago and is clinically accepted	Relatively new approach with very few approved treatments

BOX 2 Unknown factors in the mechanisms of allergen SIT

- The molecular mechanisms of how T_{reg} cells are generated *in vivo*
- How to develop improved adjuvants that can specifically induce T_{reg} cells
- The *in vivo* life span of T_{reg} cells induced by allergen SIT
- Whether there are there deleterious roles of T_{reg} cells, such as immune tolerance to tumor antigens and chronic infectious agents
- The role of resident tissue cells in immune tolerance
- The molecular mechanisms of spontaneous healing, remissions and exacerbations of allergic disease
- The local tissue events that occur during SLIT and epicutaneous SIT
- Early molecular markers and predictors to decide whether to start or stop therapy and how to measure or predict therapeutic success
- The existence of differences in the mechanisms of high-dose and low-dose allergen SIT
- The mechanisms of long-term maintenance of allergen tolerance

tolerance[16]. A study investigating a wasp venom immunotherapy used human major histocompatibility complex (MHC) class II tetramers to monitor clinical allergen tolerance and showed that this therapy led to a switch in the frequencies of the antigen-specific T cells that produced certain cytokines. In response to the wasp venom immunotherapy, there was a marked decrease in the number of IL-4–producing T cells and an increase in the number of $FOXP3^+$ and IL-10–producing antigen-specific $CD4^+$ T cells[17]. Similarly, peptide immunotherapy in allergic asthma leads to decreased T helper type 2 (T_H2) cell responses as a result of IL-10–dependent peripheral T cell tolerance. Therapy with peptides of selected T cell epitopes from the Fel d 1 major cat allergen resulted in a suppression of T cell proliferation in response to other 'linked' T cell epitopes within the same allergen[18]. As has been shown in other models, such as in suppression of germinal center reactions and in intestinal lymphoid tissues, this T cell suppression can generally take place in both secondary lymphoid organs[19,20] and in the affected tissues[21].

The investigation of human high-dose allergen exposure models has also provided key insights into the nature of T_{reg} cell responses in allergen tolerance. In nonallergic beekeepers and cat owners[7,8], T_{reg} cells specific for the major allergens in bee venom and cat saliva represent the major T cell subset present in these healthy individuals. These T_{reg} cells use numerous suppressive mechanisms, including the involvement of IL-10, TGF-β, cytotoxic T lymphocyte antigen 4 (CTLA4) and programmed death 1 (PD1)[7,22]. Supporting these findings, FOXP3 expression negatively correlates with the amount of IgE, eosinophilia and interferon-γ (IFN-γ), and the ratio of $FOXP3^+$ T cells to total $CD4^+$ T cells is significantly lower in individuals with asthma or atopic dermatitis compared to healthy individuals[23]. In addition, the expression of FOXP3 correlates with the suppressive capacity of T_{reg} cells[24].

Studies of T_{reg} cells in allergic individuals from other populations have also highlighted the key role of these cells in allergen tolerance. The induction of mucosal tolerance against dietary antigens is associated with the development of $CD4^+CD25^+$ T_{reg} cells. Children who develop clinical tolerance to milk have decreased peripheral blood mononuclear cell proliferation in response to bovine β-lactoglobulin as a consequence of an increased number of circulating $CD4^+CD25^+$ T_{reg} cells[25]. In allergic children, the number of T_{reg} cells increases during the pollen season, and these T_{reg} cells may have a role in the control of other T cell subsets that are activated by pollen allergens[26]. Both healthy and allergic individuals produce all three types of allergen-specific subsets of T cells, namely T_H1, T_H2 and $T_{reg}1$ cells, but in different proportions. Accordingly, a change in the balance between

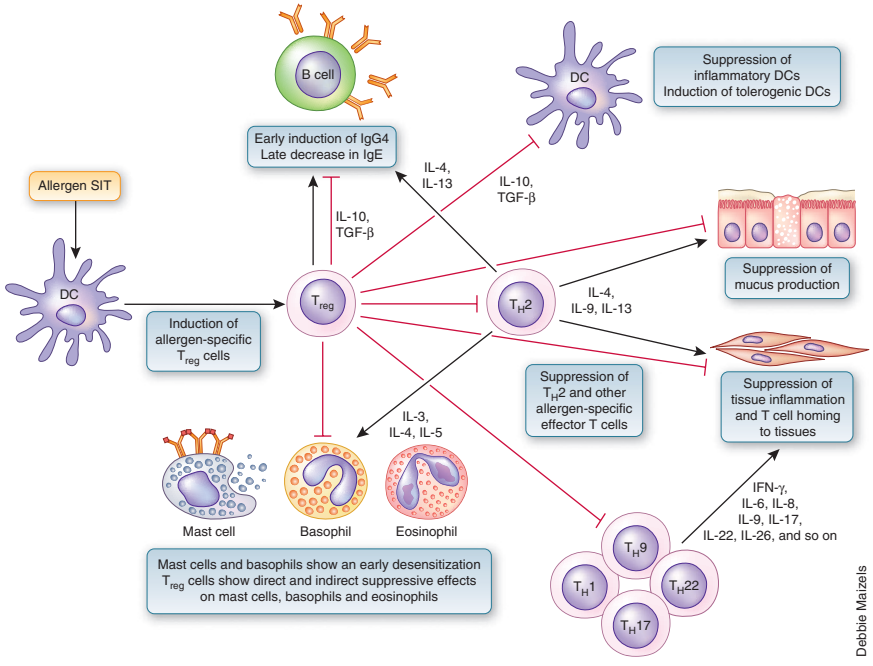


Figure 1 Mechanisms of long-term immune tolerance obtained by treatment with allergen SIT. After the first administration of the SIT vaccine, there is an early decrease in mast cell and basophil degranulation and a decreased tendency for systemic anaphylaxis resulting from early desensitization. Allergen-specific T_{reg} cells are then generated, and there is a suppression of allergen-specific T_H2 cells and other effector cells. Because of the immune tolerance of T_H2 cells, they can no longer contribute to IgE production, endothelial cell activation, T_H2 cell homing to tissues, mucus production by the epithelium or tissue migration, priming and survival of mast cells, eosinophils and basophils. IL-10 and TGF-β directly and indirectly regulate B cells and effector cells. Other T cell subsets, such as T_H1, T_H9, T_H17 and T_H22 cells, are suppressed by T_{reg} cells. Within the spectrum of changes in the immune system after allergen SIT, there is a relatively early increase in the amount of IgG4 and a late decrease in the amount of IgE. A substantial decrease in the allergen-specific IgE-to-IgG4 ratio occurs several months after allergen SIT. A decrease in the number of tissue mast cells and eosinophils and in the release of their mediators and a decrease in late-phase response is observed in the affected tissues (red lines, suppression; black arrows, induction). DC, dendritic cell.

T_H2 and T_{reg} cells, particularly in the dominant allergen-specific T cell subset, may lead to either the development of allergy or the recovery from allergic disease[7,22].

Allergen-specific IgE and IgG responses. Instead of becoming fully tolerant to allergen SIT as T cells do, B cells show a decisive skew from producing IgE to IgG4 in response to allergen SIT[27]. Allergen SIT induces a transient increase in serum-specific IgE concentrations followed by a gradual decrease in these concentrations over years of treatment[28]. In contrast, the specific concentration of IgG4 in serum has a relatively early and rapid increase in response to allergen SIT, and this concentration continues to increase during the whole duration that SIT is administered. IL-10 produced by T_{reg} cells potently suppresses allergen-specific IgE while increasing IgG4 production[12,29]. Therefore, in addition to inducing T cell tolerance, IL-10 regulates the production of specific Ig isotypes, directing the immune response toward a noninflammatory phenotype. However, this effect of IL-10 cannot explain the observed therapeutic effect of allergen SIT because the decrease in serum IgE concentration in response to SIT, occurs relatively late in the process and does not correlate with clinical improvement after treatment. The reason for the long time gap between the generation of T_{reg} cells after SIT and any changes in IgE concentrations is thought to be related to the continued production of IgE by plasma cells residing in the bone marrow that have a very long lifespan[30].

An analysis of the regulation of IgG subtypes by allergen SIT indicated that there are increases in the plasma concentrations of allergen-specific IgG1 and, particularly, IgG4 in response to SIT, and that these increases are in the range of 10- to 100-fold[31]. IgG4 competes with allergens for binding to the IgE on the Fcε receptors of mast cells and basophils and thus acts as a blocking antibody that prevents the activation and degranulation of effector cells[32]. In addition, some distinct features of IgG4 suggest that it may have an anti-inflammatory role. IgG4 antibodies are dynamic molecules that exchange fragment, antigen binding (Fab) arms by swapping heavy-light chain pairs between IgG4 molecules with different specificities. This process results in the production of bi-specific antibodies with a substantially decreased capacity for crosslinking because they are functionally monomeric[33]. In addition, the IgG4 hinge region has specialized structural features that result in a lower affinity for certain Fcγ receptors, and IgG4 does not fix complement and can inhibit immune-complex formation by other antibody isotypes[34].

Suppression of mast cells, basophils and eosinophils. The thresholds for mast cell and basophil activation and decreased IgE-mediated histamine release are efficiently modulated by allergen SIT[35]. Several molecular mechanisms have been proposed for how T_{reg} cells and anti-inflammatory cytokines affect mast cells and basophils. IL-10 suppresses IL-5 production through human T_H2 cells, reduces proinflammatory cytokine release from mast cells and downregulates eosinophil function and activity[36]. Furthermore, through direct contact between T_{reg} cells and mast cells, T_{reg} cells inhibit Fcε receptor I (FcεRI)-dependent mast cell degranulation[37]. Mast cells also seem to have a role in immune tolerance and are not simply enhancers of allergic inflammation; for example, mast cells downregulate allergic inflammation in ultraviolet light–induced skin injury and venom-induced tissue damage models in which IL-10 has a key role[38,39]. T_{reg} cells have been reported to be suppressive in various models of eosinophilic inflammation, including inflammation induced by schistosome infection and asthma-like lung inflammation in mice[40]. In addition, decreased numbers of eosinophils and decreased concentrations of eosinophil chemoattractants and eosinophil mediators have been observed in the nasal mucosa as a long-lasting effect of allergen SIT[41].

Vaccine development for allergen SIT and immunomodulation

Although it has been performed in the clinic for the past 100 years, allergen SIT faces several problems related to its limited efficacy, side effects, low patient adherence and the high costs resulting from the long duration (3–5 years) of treatment required. The main approaches to improve the efficacy and safety of vaccine-based allergen SIT can be classified into six groups (**Table 2**).

The first approach is targeting T cells to induce T cell tolerance and bypassing IgE binding to avoid IgE-mediated side effects[42]. The conformation dependence of B cell epitopes and the linearity of the amino acid sequence of T cell epitopes in the three-dimensional structure of an allergen have been targeted using allergen fragments, fusions, hybrids and chimeras[42–45]. The prototype of this approach is peptide immunotherapy that uses linear T cell epitope peptides[46–49]. All of these T cell–targeting approaches enable higher doses of allergens to be sequentially administered, which is required to induce T cell tolerance without risk of anaphylaxis[42,50].

The second approach is the use of recombinant allergens or their mixtures to partially reconstitute an allergen extract. A study that tried to reconstruct a native grass pollen allergen extract using a mixture of five recombinant allergens was effective in reducing symptoms and the need for symptomatic medication in patients with grass pollen allergy[31]. All treated subjects developed high allergen-specific IgG1 and IgG4 antibody responses. During the study, seven systemic reactions were observed in patients from a total of 1,479 injections. Relatively mild local side effects related to treatment were observed in 10.7% of the patients that were actively treated with the recombinant allergens and in 5.9% of the patients receiving placebo. There have been 15 clinical trials that used recombinant allergens and showed greater clinical efficacy of the allergens compared to placebo over the last decade. However, licensing all these products may not be cost effective, particularly for minor allergens that do not cause allergic reactions in a substantial number of people. Recombinant vaccines for grass pollen, birch pollen and house dust mites have been the major focus in these trials.

The third approach is to physically couple allergens to stimulators of the innate immune response. This area is open to the possibility of future developments, as there are infinite numbers of possible combinations owing to the existence of multiple immune stimulators and methods for coupling[51–55]. Notably, the time, intensity and tissue location of stimulation of the innate immune response by the allergen are key to the induction of tolerance or immune activation. For example, house dust mites can activate Toll-like receptor 4 (TLR4) in airway structural cells to induce asthma-like inflammation in mice[56]. However, it should also be noted that natural exposure to house dust mites and other aeroallergens involves exposure to innate immune response–stimulating substances that activate TLR4.

The fourth approach is varying the routes of vaccine administration. A meta-analysis of double-blind, placebo-controlled trials of sublingual immunotherapy (SLIT) indicated that SLIT showed clinical efficacy and had a treatment benefit of approximately half of that achieved by subcutaneous allergen SIT[57]. Sustained disease-modifying effects of this type of therapy have been shown in large-scale randomized, double-blind, placebo-controlled trials in adults and in children[5,58]. Although the magnitudes of the changes in most clinical

Table 2 Vaccine development for allergen SIT

Type of vaccine or approach	Developmental status	Comments	References
	Bypassing IgE and targeting T cells		
Fusion of major allergens and chimeric allergens	Effects in human cell cultures and mouse models	Major allergens or their fragments are fused and expressed as a single recombinant protein. T cell reactivity is preserved, and IgE binding is attenuated. Prevention of IgE production has been shown in mice	43,44
Hypoallergenic hybrid molecules	Effects in human cell cultures and mouse models	Hybrid proteins derived from the Der p 1 and Der p 2 allergens reduce IgE reactivity and induce greater T cell proliferation	121
Fragments of major allergens	A multicenter clinical trial was reported in 2004. The approach has not been pursued further	Use of fragments of a major allergen (Bet v 1) that do not bind IgE. IgE binding is attenuated, and T cell reactivity is preserved	45
Peptide immunotherapy	Several clinical studies have been performed. Long peptides (27 amino acids in length) have been associated with side effects. Short peptides have been shown to be safe and tolerable in people allergic to cats in a dose-ranging phase 2a clinical trial (NCT00867906)	T cell epitope peptides (Fel d 1, Api m 1) that do not bind IgE and induce T cell tolerance have been used in cat and bee venom allergies	46–49
Unrefolded native or recombinant allergens	Several ongoing clinical trials have reported promising results	Major recombinant allergens (Api m 1, Bet v 1) that are not properly refolded and lack their native conformation have been used. IgE binding is abolished and T cell reactivity is protected	50
Polymers of major allergens	A multicenter clinical trial was finalized several years ago, and this approach has not been pursued further	A trimerized major allergen (Bet v 1) has been used. Mast cell and basophil degranulation is attenuated, and T cell reactivity is preserved *in vitro*	45
	Reconstitution of the natural extract with multiple recombinant allergens		
Mixtures of several major recombinant allergens	One clinical trial reported promising results. A double-blind, placebo-controlled, dose-response study is under evaluation	Phl p 1, Phl p 2, Phl p 5a, Phl p 5b, Phl p 6 were used in combination as a mixture of five recombinant grass pollen allergens. A reduction in symptoms and the need for symptomatic medication was observed in people who were allergic to grass pollen	31
	Allergens coupled to adjuvants		
GpG oligonucleotide-conjugated allergens	A large multicenter clinical trial did not reach its endpoints	A TLR9-triggering CpG oligonucleotide fused to a major ragweed allergen Amb a 1 has been tested	51
Allergens coupled to virus-like particles	A rapid induction of high IgG antibody titers was observed in healthy human volunteers	Highly repetitive virus capsid-like recombinant particles coupled to the house dust mite major allergen Der p 1 have been tested	52
Carbohydrate-based particles	Effects shown in mouse models	Carbohydrate-based particles bound to the allergen rPhl p 5b induced strong antibody and cytokine responses	53
Hypoallergenic vaccine based on allergen-derived peptides fused to hepatitis B PreS antigen	Effects shown in mouse models	Recombinant fusion proteins showed reduced allergenic activity with lowered basophil activation. There was no IgE reactivity to the fusion protein	54
Monophosphoryl lipid A (MPL) formulated with allergoid	Clinical trials have reported safety and efficacy. A phase 3 study has been completed (NCT00414141)	T_H1-inducing adjuvant monophosphoryl lipid A (MPL) facilitated short-term SIT together with a grass pollen allergoid	55
	New routes of administration		
Intralymphatic vaccination	A clinical trial has reported safety and efficacy. A phase 3 study is ongoing (NCT01166269)	Allergen SIT vaccines are administered directly into inguinal lymph nodes with the aim of delivering high amounts of allergens into secondary lymphatic organs	63
Epicutaneous vaccination	A clinical trial in grass-pollen–induced rhinoconjunctivitis showed safety and efficacy. A phase 2 study is ongoing in children with a peanut allergy (NCT01197053)	High numbers of antigen-presenting cells (Langerhans cells) are delivered to a nonvascularized area. The method is safe, needle free and has the potential for self administration	64
	Fusion with immune response modifiers		
Targeting FcγRII	Effects have been shown in human cell cultures and mouse models	Fusion of allergens to human Fcγ has been reported to inhibit allergen-induced basophil and mast-cell degranulation by crosslinking Fcγ and FcεRI receptors	65,66
Modular antigen translocation (MAT) vaccines	A clinical trial has been finalized and showed safety and efficacy with evidence of immune regulation	The coexpression of major recombinant allergens together with the transactivator of transcription (Tat) peptide and truncated invariant chain peptide can target antigens to the MHC II molecules in the trans-golgi compartment	68
	Combined treatment with immune response modifiers		
Pretreatment with mAbs to IgE before SIT	Several investigator-initiated clinical trials are ongoing to reduce SIT-induced side effects and to enable relatively rapid dose increases and the use of relatively high doses of therapy	Significantly fewer systemic allergic reactions were observed, and more patients were able to reach the target maintenance immunotherapy dose	69

and immunological parameters have been modest or no changes have been observed in response to SLIT, the immunological mechanisms of SLIT seem to be similar to those of subcutaneous allergen SIT. The reduced treatment benefits and modest changes in immunological markers observed with SLIT suggest that there is still room to improve this method of vaccine delivery. Multiple mechanisms of immune tolerance are induced by SLIT that involve T_{reg} cells, IL-10 production and increased numbers of sublingual FOXP3-expressing T cells. In addition, increased concentrations of blocking IgG4 and of IgA antibodies that show inhibitory activity for IgE-facilitated binding of allergens to B cells have been observed after SLIT, similar to what is seen after subcutaneous allergen SIT[59,60]. Recently, allergen-specific $FOXP3^+$ T_{reg} cells have been found in human lingual and palatine tonsils in humans, and these cells may participate in oral allergen tolerance and SLIT[61]. It is possible that sugar-modified antigens can be used to induce oral tolerance, and a C-type lectin receptor, SIGNR1 (also known as CD209b), has recently been shown to condition dendritic cells to induce tolerance in the gastrointestinal lamina propria in a model of food-induced anaphylaxis[62].

Recently the intralymph node and epicutaneous routes of vaccine delivery have been tested. Both these routes showed a similar efficacy as subcutaneous immunotherapy injections in the treatment of grass pollen allergy, but fewer applications and lower total doses of allergen were required using these two routes[63,64]. Intralymphatic vaccines have been shown to induce T cell responses that are associated with strong cytotoxic activity and IFN-γ production, which are key in long-term protection against viral infections and tumors.

The fifth strategy is the fusion of allergens to immune modifiers. FcγRIIb is an immune tyrosine-based inhibitory motif-containing receptor[65]. The coaggregation of FcεRI and FcγRIIb inhibits FcεRI signaling, so one strategy that has been tested in the treatment of allergy is the fusion of FcγRIIb to allergens to downregulate downstream allergen-specific immune responses. In a similar approach, the fusion of allergens to human Fcγ suppressed allergen-induced degranulation of basophils and mast cells by crosslinking Fcγ and FcεRI[65,66]. Recently, the major cat allergen Fel d 1 was cloned and expressed together with a human immunodeficiency virus protein, TAT-derived membrane translocation domain, and a truncated peptide of the invariant chain (modular antigen translocation (MAT)-Fel d 1)[67]. This MAT–Fel d 1 vaccine is efficiently internalized and potently presented to T cells by antigen-presenting cells, and the vaccine induces T cell responses at doses that are approximately 100 times lower than those at which the native allergens are present. In a double-blind, placebo-controlled clinical trial, the MAT–Fel d 1 vaccine in alum adjuvant was administered in three increasing doses (1 μg, 3 μg and 10 μg) into the inguinal lymph nodes of patients with cat allergies at 4-week intervals. The vaccine showed a good safety profile, and after treatment, individuals who were allergic to cats became clinically tolerant to nasal challenge of cat dander extract, which was observed in parallel with increased concentrations of serum IgG4 in these individuals[68].

In addition to physical fusion, conventional and new methods of allergen SIT may also be combined with immune-response modifiers. For example, a monoclonal antibody (mAb) to IgE combined with allergen SIT has been evaluated in several studies[69], and this combination treatment resulted in a significant decrease in the risk of anaphylaxis caused by rush immunotherapy (which uses rapid dose increases to reach the maintenance therapeutic dose as quickly as possible) and improved rescue medication scores (therefore decreasing the need for a rescue medication to suppress the symptoms) of allergen SIT with a good safety profile[69,70]. Combination strategies with biological immune-response modifiers are expected to substantially expand the treatment scope of allergen SIT. Although there has been some progress in improving the efficacy and safety of allergen SIT, which is the only approach currently being investigated that could cure allergic diseases, further modifications could still be made to this mode of therapy, which are hoped to improve the application of SIT to allergic diseases as well as to other diseases related to immune dysregulation.

Biological immunotherapeutics in allergy and asthma

Developments in understanding the immunological mechanisms of allergy and asthma have enabled the identification of many potential therapeutic targets (**Box 3**). Because of the complexity and redundancy of the immune mechanisms that are involved in asthma and allergy, it is possible that the use of a single biological might be effective in specific subgroups of asthma and atopic dermatitis, such as for well-defined endotypes that can identify those individuals who will benefit most from a particular treatment. Therefore, many new treatments that target a single mediator or receptor are currently in clinical development or preclinical investigation (**Table 3**). However, given the complex clinical spectrum of these diseases, it is improbable that any of these biologicals alone will have a major effect in a clinical trial testing a population of individuals that are heterogeneous for the allergic disease being studied or for asthma who have not been classified into specific phenotypes or endotypes. When a new drug is developed to target a very specific molecular mechanism, it is expected that it will function only in a specific asthma endotype. It is anticipated that endotype-tailored treatment (known as 'stratified medicine') could lead to measurable improvements in the health economics of asthma, but more importantly, it may enable disease management to be optimized for individual patients. Currently, combinations of biologicals or the sequential use of two or more biologicals are being considered as viable therapeutic strategies, as reported recently in a study that used a treatment combining mAb to IgE and mAb to B cell CD20 (ref. 71). However, the combination of two biologicals is prohibitively expensive, and it is practically impossible to combine biologicals in a clinical trial setting before one of them gets full approval. This problem needs to be solved before combination strategies of two unapproved biologicals can be developed, which may take several years.

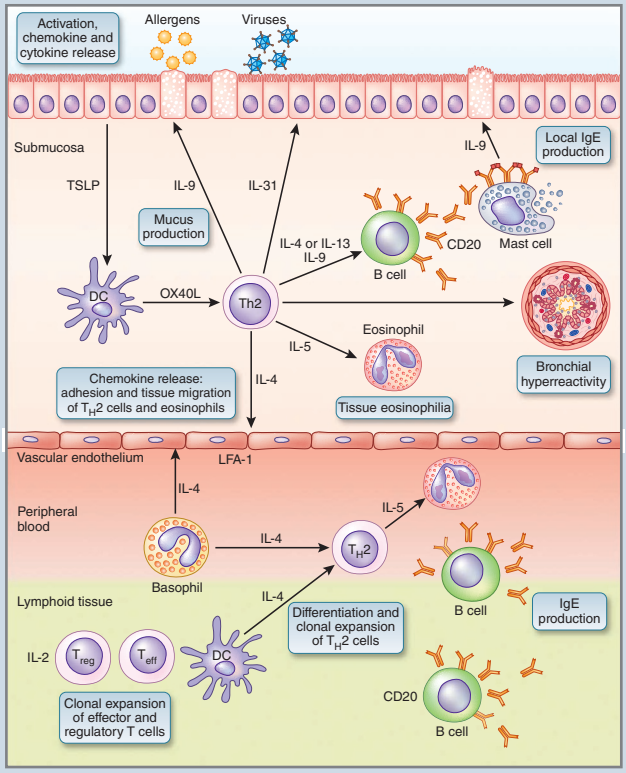
Treatment strategies focused on IgE

Several clinical studies to test different humanized mAbs that bind to the Fc portion of the IgE molecule have been performed, and one of the mAbs tested, omalizumab, was shown to be an effective treatment in patients with poorly controlled, moderate to severe allergic asthma or allergic rhinitis[72–74]. Omalizumab decreases the serum concentration of free (unbound) IgE and the expression of the high-affinity IgE receptor FcεRI on several cell types[72]. Soluble immune complexes of IgE and mAb to IgE are subsequently cleared by the reticuloendothelial system. Omalizumab binds to the same site that IgE molecules use to attach to FcεRI and cannot crosslink cell-surface–expressed IgE[75].

Clinical studies of mAb to IgE have improved our understanding of the multiple roles of IgE in the development of the allergen-specific immune response and inflammation. Omalizumab reduces the expression of FcεRI on basophils, dendritic cells and monocytes within 7 d of administration[72]. Omalizumab treatment reduces the number of eosinophils in the blood of individuals with seasonal allergic rhinitis and asthma, as well as the number of sputum eosinophils

BOX 3 Targets for new immunomodulatory drugs in asthma

- Epithelial cell activation and the proinflammatory cytokines and chemokines that they produce, which induce inflammation and contribute to T_H2 responses: TNF-α, IL-13, TSLP and IL-31, IL-33 (refs. 89,90,97,98,105,131,132, 134,137)
- Epithelial apoptosis and shedding in eczema and asthma[143,144]
- The T_H2 response: IL-4, IL-5, IL-9, IL-13, IL-25 and IL-33 (refs. 88–91,101,105,123,124,126–129,131–134)
- Eosinophilia: IL-5, IL-25 and IL-33 (refs. 91,105,127–129,133)
- Local and systemic IgE production: IL-4, IL-13, CD20 and IgE[88–90,119,123,124,126,131,132,145]
- Crosslinking of IgE receptor FcεRI on the surface of mast cells and basophils and their degranulation[146,147]
- Smooth muscle and myofibroblast activation and bronchial hyperreactivity: IL-4, IL-9, IL-13, IL-25, IL-33 (refs. 89,90, 101,105,131,132,134)
- Survival and reactivation of migrating inflammatory cells and their interaction with resident tissue cells and other inflammatory cells: IL-2, IL-4 (refs. 88,122–124,126)
- Cell migration and chemokines[110–112,139–141]
- Other effector T cell subsets, such as T_H9, T_H17 and T_H22 cells[148–150] (see illustration)



in patients with asthma. In addition to its effects on mast cells and basophils that are related to decreasing the concentrations of free serum IgE, therapy with mAb to IgE causes a rapid decrease in FcεRI expression on the surface of dendritic cells, suggesting that circulating IgE regulates the expression of this molecule on dendritic cells[76]. A significant number of patients treated with omalizumab who had allergic asthma and rhinitis had a positive response to treatment and were able to decrease or discontinue their use of inhaled corticosteroids[73]. Studies of therapies using mAb to IgE in children have been limited, but a recent study showed that children, adolescents and young adults with atopic asthma living in the inner city who were treated with omalizumab had reductions in the number of asthmatic episodes, particularly if they were first sensitized to cockroach antigens[74]. However, two current caveats to the use of treatment with mAb to IgE are its unexpected efficacy in some cases without any reasonable explanation of the underlying molecular mechanisms, as well as considerations of cost effectiveness. In a recent study, treatment with omalizumab reduced histamine release from peripheral blood leukocytes stimulated with cat allergen *in vitro*; however, paradoxically, histamine release increased twofold after omalizumab-mediated basophil stimulation[77]. The cost effectiveness of omalizumab has been evaluated and has been shown to be low in patients with moderate to severe allergic asthma compared to the clinical benefits of the drug. Given this evaluation, one possible approach to use omalizumab is a relatively late treatment choice as an add-on therapy[78].

Although there have been no detailed double-blind, placebo-controlled studies on the subject, several reports have suggested that treatment with mAb to IgE is also beneficial for patients suffering from other IgE-related conditions, such as atopic dermatitis, peanut allergy, latex sensitivity, chronic urticaria or allergic bronchopulmonary aspergillosis. In allergic bronchopulmonary aspergillosis, omalizumab has been shown to eliminate the need for treatment with steroids[79]. Treatment with mAb to IgE has been reported to be effective in several cases of recurrent idiopathic angioedema[80] as well as in unprovoked anaphylaxis in patients with systemic mastocytosis[81]. In most patients with Churg-Strauss syndrome, a type of autoimmune vasculitis, severe asthma and upper respiratory symptoms persist, requiring continuous therapy. Treatment with mAb to IgE was shown to improve asthma and decrease the eosinophil blood count in individuals with this syndrome[82].

Cytokine inhibitors

The inflammatory processes that underlie asthma and allergy are coordinated by a cytokine network. Modulating this network using new biological molecules has continuously been attempted in clinical trials for almost two decades. Some of these studies have been unsuccessful, and some trials have had to be discontinued, even for agents that have been approved for other indications, suggesting that there are substantial hurdles to be overcome for the clinical use of cytokine inhibitors to treat asthma and allergy. Some cytokines show redundancy in their ability to generate or suppress T_H cell subsets and T_{Reg} cells in addition to their roles in disease pathogenesis, which may indicate that agents targeting a single cytokine may not be effective. Despite this redundancy, therapies using cytokine inhibitors may provide a way to elucidate the role of individual cytokines in the pathogenesis of human disease, although it should also be acknowledged that the complexity of the inflammatory networks might make

Table 3 Immune response modifiers that can be targeted for the treatment of allergy and asthma

Target	Molecule	Developmental Status	Comments	References
		Approaches that target cytokines		
IL-2	mAb blocking IL-2 receptor α (CD25) (daclizumab)	Phase 2 has been completed, and efficacy has been observed for asthma	Inhibits the proinflammatory cytokine generation by IL-2R blockade on T cells. No negative effects have been observed on T_{reg} cell generation *in vivo*	122
IL-4	Humanized mAb blocking IL-4 (pascolizumab)	Phase 2 has been completed	Approaches that only targeted IL-4 were not effective, and developments have been halted	123
	Mutated IL-4 (pitrakinra): blocks IL-4Rα, which is common in IL-4 and IL-13 receptors	Reduced allergen responses in asthma and is in clinical trials. Phase 2 has been completed. Effective in a monkey model of asthma	Targeting of IL-4 and IL-13 at the same time showed some efficacy	124
	Blocking mAb to IL-4Ra, also blocks IL-13 (AMG-317)	Phase 2 has been completed, and some efficacy was observed		88
	Inhaled oligonucleotide against IL-4Ra, also blocks IL-13 (AIR-645)	Inhibitory effects in allergen challenge. In clinical development. Phase 2 has been completed		125
	Soluble recombinant human IL-4 receptor (altrakincept)	Phase 1 and 2 clinical trials showed efficacy for moderate asthma. A phase 3 trial did not confirm this efficacy		126
IL-5	Humanized blocking mAb to IL-5 (mepolizumab)	Effective in severe asthma with sputum eosinophilia and exacerbations in clinical trials. Phase 2 has been completed. Recruiting for phase 3 trial	Can be effective in an eosinophilic asthma endotype. Long-term inhibition of pulmonary eosinophilia and airway hyperresponsiveness was observed. Weak correlation with clinical symptoms, although peripheral eosinophila decreased	127
	Humanized blocking mAb to IL-5 (SCH-55700)	Reduced the number of circulating eosinophils in asthmatics and there was an improvement in baseline FEV1. Phase 2 is complete		91
	IL-5a receptor mAb (MEDI-563)	Effective in asthma. Phase 2 is complete		128
	Humanized blocking mAb to IL-5 (reslizumab)	Under development for the treatment of pediatric eosinophilic esophagitis and hypereosinophilic syndrome. Phase 2 has been completed for subjects with poorly controlled asthma	Reslizumab reduced intraepithelial esophageal eosinophil counts in children and adolescents with eosinophilic esophagitis. Improvements in symptoms were observed in all treatment groups, including placebo	129,130
IL-9	Blocking mAb to IL-9 (MEDI-528)	Small effect in phase 2 studies	May only be effective in a subgroup of patients in which IL-9 has a dominant role in disease pathogenesis	101
IL-13	Soluble IL-13Rα2 Fc fusion protein	Preclinical study in mice	IL-13Rα2 and IL-10 coordinately suppressed airway inflammation, airway hyperreactivity and fibrosis	131
	Blocking mAb to IL-13 (CAT-354)	Phase 1 clinical trial was completed investigating the pharmacokinetics. Phase 2 trial in patients with uncontrolled asthma despite optimal treatment has been completed	Reduced airway hyperreactivity, airway eosinophilia and esophageal eosinophilia in a mouse model of respiratory and esophageal inflammation induced by intratracheal human IL-13	89
	Blocking mAb to IL-13 (lebrikizumab)	Improvement in lung functions in asthma. Phase 2 has been completed	Was effective in an endotype that had high levels of periostin expression	90
	Blocking humanized mAb to IL-13 (IMA-638)	Phase 1 and 2 trials have been completed	Dose-dependent inhibition of the allergen induced late responses and improved total nasal symptom scores in a subgroup with high late-phase nasal IL-13 concentrations at time of screening	132
IL-17	Blocking mAb to IL-17	Preclinical development for severe asthma in mice		102
	Blocking mAb to IL-17RA	Preclinical development for severe asthma in mice		103
IL-25	Blocking mAb to IL-25	Preclinical development in mice	Blocking IL-25 in an experimental model of allergic asthma prevented airway hyperreactivity, as well as a reduction in the IL-5 and IL-13 concentrations and eosinophilic inflammation	133
IL-31	Blocking mAb to IL-31	Tested in mice	Reduced scratching behavior in an atopic dermatitis model	134
IL-33	Blocking mAb to IL-33	Preclinical development in mice	IL-33 is an IL-1–like cytokine that can activate mast cells and T_H2 cells and can induce production of T_H2 cytokines	105
TSLP	Blocking mAb to TSLP, TSLPR-Ig	Preclinical development in mice		135
TNF-α	Infliximab, chimeric mAb	Phase 2 for asthma has been completed	Targeting TNF-α has been considered to be a promising option, especially for the treatment of non-controlled asthma. Some studies have been stopped because of side effects, some studies have been completed with some efficacy, and some studies did not show any efficacy. May not be pursued because of unfavorable risk-benefit ratio. Essential to monitor and screen for infections	97

(Continued)

Table 3 (Continued)

Target	Molecule	Developmental Status	Comments	References
	Adalimumab, human mAb	Phase 2 study for asthma was withdrawn before enrollment		136
	Golimumab, human mAb	Phase 2 for asthma has been completed		137
	Etanercept, soluble TNF receptor fusion protein	Phase 2 study for moderate to severe and refractory asthma showed improved airway hyperresponsiveness, FEV1 values and overall asthma symptom scores. Phase 2 has been completed		98
Light	Fusion protein blocking the receptor	Preclinical development in mice		99
		Cell adhesion and costimulation		
LFA-1	Humanized mAb against CD11a, LFA-1 (efalizumab)	Phase 2 has been completed. Drug has been withdrawn from market	Reduced cellular infiltrate in atopic dermatitis lesions. Increased risk of infection, reactivation of latent and chronic infections and increased risk of progressive multifocal leukoencephalopathy	138
OX-40 ligand	Blocking mAb to OX40L	In preclinical development in mice	Inhibited T_H2-type immune responses induced by TSLP	107
		Chemotaxis		
CCR3	Small-molecule antagonists	Toxicology problems in rats		139
CCR4	Blocking antibody (AMG-761)	Preclinical development in mice		140
	Small-molecule antagonist (K-327)	Preclinical development in mice		141
CXCR2	Small-molecule antagonists (SCH527123)	Phase 2 studies have been completed	Effective at inhibiting neutrophil recruitment, mucus production and goblet cell hyperplasia in ozone-induced airway inflammation. Was well tolerated	110
DP1	Selective agonist for DP1 receptor (BW245C)	Regulates immune and skin allergic responses in mouse models	The balance between DP1 and DP2 receptors is key in basophil responses	111
CRTH2	Selective CRT_H2 antagonist (OC000459)	Phase 2 studies have been completed for asthma	Inhibitor of mast-cell–dependent activation of T_H2 lymphocytes and eosinophils	142
		B cells		
CD20	Humanized mAb that binds to CD20 on B cells	Effective in atopic dermatitis in an investigator-driven clinical study	CD20 has a role in the regulation of human B cell activation, proliferation and differentiation	119
		IgE		
IgE	Humanized mAb that binds to Fc portion of human IgE (omalizumab)	Approved for the treatment of patients with persistent severe allergic asthma despite administration of optimized controller therapy	Safety and efficacy of this mAb in allergic conditions other than asthma and in pediatric patients below the age of 12 should be clarified	72–74

these individual roles difficult to decipher. In addition, some of these biological modifiers could be possible candidates to augment the effects of allergen SIT in combination strategies.

Strategies using mAb to IL-2. It is generally thought that strategies that block IL-2 pathways could adversely affect $CD25^+FOXP3^+$ T_{Reg} cell populations, which also rely on IL-2 signaling for their expansion and survival. In addition to promoting the proliferation and survival of recently activated effector T cells, IL-2 is also key in T_{Reg} cell homeostasis, thymic development and suppressive function of T_{Reg} cells. Daclizumab is a humanized monoclonal antibody that binds to the IL-2 receptor α chain CD25 on activated lymphocytes, inhibits IL-2–induced proliferation and reduces the production of T_H2 and T_H1 cell cytokines by activated T cells. A phase 2 study in patients with moderate to severe asthma showed that daclizumab improved pulmonary function, reduced asthma symptoms and the need for 'rescue' medication, increased the intervals between asthma exacerbations and reduced blood eosinophilia concentrations and the amount of serum eosinophil cationic protein[83,84]. The therapeutic benefit of daclizumab treatment in patients with more refractory asthma was more pronounced. The issue of whether blocking IL-2 signaling adversely affects T_{reg} cell development and function was assessed in a heart transplantation study, which showed that daclizumab treatment did not interfere with T_{reg} cell generation and had a beneficial effect on heart allograft survival[85].

Strategies targeting IL-4. IL-4 induces IgE isotype switching and promotes the differentiation of naive $CD4^+$ T lymphocytes into T_H2 cells, as well as the subsequent release of increased amounts IL-4, IL-5 and IL-13 by T_H2 cells. IL-4 may have a role in the expression of adhesion molecules and chemokines that promote the migration of T_H2 cells and eosinophils into tissues, as well as in the development of myeloid dendritic cells. Several clinical trials have evaluated the efficacy and safety of a recombinant human soluble IL-4 receptor (IL-4R) and mAbs to IL-4, but no significant improvements were noted in symptoms or asthma exacerbations using treatments with either of these two agents. These findings suggested that targeting IL-4 on its own does not seem to have clinical benefit and led to the discontinuation of the further exploration of therapies that target IL-4. One of the primary reasons why this approach failed could be the high redundancy of IL-4 and IL-13 signaling. Therefore, it may be necessary to block both IL-4 and IL-13 to see any efficacy for this type of therapy. The receptor of IL-4 and IL-13 consists of two chains, IL-4Rα (CD124) and the common γ chain. IL-13R consists of two subunits, IL-13Rα1 and IL-13Rα2, and IL-4 and IL-13 signaling occurs through the receptor complex type II that consists of the IL-4Rα and IL-13Rα (ref. 86).

A mutated IL-4 protein that inhibits the effects of both IL-4 and IL-13 through its ability to block IL-4Rα has been generated, and clinical studies testing therapies using this agent are currently under way[87]. A mAb to IL-4Rα that blocks both the IL-4 and IL-13 receptors may have the same potential as the mutant IL-4 protein for blocking the effects of both of these cytokines[88].

Strategies using mAb to IL-13. IL-13 is as crucial as IL-4 in the production of IgE, and studies have indicated that this cytokine induces airway hyperresponsiveness, epithelial activation, mucous production, chemokine secretion and changes in airway remodeling. Studies in mouse and monkey models of asthma have shown that treatment with mAb to IL-13 leads to a reduction in lung inflammation. Various humanized mAbs specific for human IL-13 have been, and are currently, in development. A phase 1 clinical trial showed that increasing single doses of intravenously administered mAbs to IL-13 in mild asthma were well tolerated at all doses, and there were no safety concerns associated with this treatment[89]. A recently reported phase 2 trial of a mAb to IL-13 (lebrikizumab) provided convincing evidence that this biological could be effective in treating certain disease subgroups that have been selected on the basis of a biomarker. Notably, this mAb to IL13 showed significant efficacy in a patient group that expressed high amounts of the protein periostin but did not have any effect in patients with low amounts of periostin, which is what would be predicted based on knowledge of the functions of periostin. IL-13 induces periostin, and periostin has a role in type I collagen synthesis, fibrosis and remodeling in the lungs[90]. Therefore, reducing the amount of free serum IL-13 or blocking IL-13 signaling in individuals with low amounts of periostin would not be expected to have any beneficial effect, as alternative pathogenic pathways that are not downstream of periostin are probably operating in these individuals.

Strategies using mAb to IL-5. Eosinophilia in the lung and circulation are hallmarks of asthma, and IL-5 is a key cytokine for eosinophil differentiation and survival. For this reason, there has been intensive focus on strategies using mAb to IL-5 for the treatment of asthma. A decrease in the number of serum eosinophils was noted in subjects with severe persistent asthma treated for 90 d with reslizumab, a humanized mAb to IL-5 (ref. 91). No significant sustained changes in forced expiratory volume in 1 s (FEV1), asthma symptom scores or the percentage of sputum eosinophils were noted with any dose of reslizumab. Studies in patients with mild atopic asthma showed that the humanized mAb to IL-5 mepolizumab decreased extracellular matrix protein remodeling and the percentage of airway eosinophils. Treatment with mepolizumab decreased serum eosinophilia by 100%, whereas it decreased airway eosinophils to a lesser extent, by 55%[92]. Similar to reslizumab, mepolizumab treatment did not affect the clinical measures of asthma, including airway hyperresponsiveness, FEV1 and peak flow measures. Recent trials of mAb to IL-5 in patients with prominent sputum eosinophilia and severe asthma that is refractory to existing therapies showed that the treatment significantly reduced asthma exacerbations[93]. Notably, in these trials, only 5% of the patients showed this clinical improvement and fit into a group that had eosinophils comprising greater than 3% of their total sputum cells, suggesting that it may be possible to define an asthma endotype that is responsive to mAb to IL-5, similar to the endotypes observed for periostin and lebrikizumab. If, similar to mAb to IL-5 and IL-13, a drug is only successful for a small percentage of affected individuals, it is now a matter of debate whether this factor would influence the likelihood of approval of the drug. In addition, treatment with mAb to IL-5 has shown promising results in patients with hypereosinophilic syndrome[94], nasal polyps[95] and eosinophilic esophagitis[96].

Strategies to target tumor necrosis factor-α. Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine that has been implicated in many aspects of the airway pathology in asthma, particularly in refractory and severe cases. Clinical trials have shown improvement in lung function, airway hyperresponsiveness and quality-of-life symptom scores and reduction in the exacerbation frequency in patients with asthma who were treated with mAb to TNF[97,98]. Treatment with etanercept, a soluble TNF receptor (TNFR) fusion protein, also improved airway hyperresponsiveness, FEV1 values and overall asthma symptom scores in a phase 2 study for moderate to severe and refractory asthma[98]. Although promising results have been obtained using these agents, because of their association with an increased risk of infection, particularly tuberculosis and malignancy, strategies that target TNF will probably not be enthusiastically pursued for general treatment of asthma.

The TNF superfamily consists of many membrane-bound and soluble proteins with proinflammatory effects in the innate and adaptive immune responses. A recent preclinical study showed that using a fusion protein to block the TNF ligand superfamily member 14, LIGHT, which is expressed on many immune cells such as activated T cells, monocytes and macrophages, reduced airway hyperresponsiveness, lung fibrosis and smooth muscle hyperplasia in mouse models of chronic asthma, despite showing little effect on airway eosinophilia in these models. LIGHT may be therapeutically targeted to prevent asthma-related airway remodeling, as LIGHT-deficient mice also showed an impairment in fibrosis and in smooth muscle accumulation[99]. However, given the safety concerns that already exist with therapies targeting TNF-α, it is probable that similar issues also exist for targeting LIGHT and other TNF-family molecules.

Other approaches using cytokine inhibitors. A number of other therapeutic approaches to target cytokines are in various stages of preclinical and clinical development and testing. A newly described population of T cells called T_H9 cells, which produce IL-9 and IL-10, have been proposed to have a role in allergic inflammation[100]. IL-9 is secreted by $CD4^+$ T_H2 cells, eosinophils, mast cells and neutrophils. It inhibits cytokine production by T_H1 cells, induces chemokine and mucous secretion by bronchial epithelial cells and promotes the proliferation of mast cells. In a recent clinical trial, an mAb to IL-9 did not to meet the clinical endpoints; however, it did show an acceptable safety profile, and findings from the trial were suggestive of clinical activity in subjects with mild to moderate asthma[101]. Approaches that target IL-17, IL-25, IL-31 or IL-33 may be useful in some endotypes of asthma and are currently in preclinical development[102–105]. However, positive results from preclinical studies do not necessarily mean that these approaches will work in the setting of human asthma, and in addition, specific endotypes that might be targeted by these cytokines have not been elucidated in humans.

Thymic stromal lymphopoietin (TSLP) is an essential cytokine for the initiation and development of allergic inflammation. Treatment with TSLP diverted airway tolerance against ovalbumin to T_H2 sensitization and inhibited the generation of ovalbumin-specific inducible T_{reg} cells in an asthma-like mouse model[106]. A soluble TSLP antagonist, TSLPR immunoglobulin, was shown to reduce the severity of allergic disease by blocking TSLP signaling and by regulating pulmonary

dendritic cells. The tumor necrosis factor receptor superfamily member 4 (OX40) ligand (OX40L) is a key regulator of TSLP-mediated T_H2 responses. Preclinical studies in a mouse model of asthma with an mAb that blocked OX40 signaling showed promising results, with substantial inhibition of the immune responses induced by TSLP in the lung and skin, including decreased T_H2 cell infiltration, cytokine secretion and IgE production. OX40L-blocking antibodies have also been shown to inhibit antigen-driven T_H2 inflammation in mouse and nonhuman primate models of asthma[107].

Targeting cell adhesion, co-stimulation and chemotaxis

Chemokine receptors, which are G-protein–coupled receptors, can be therapeutically targeted by small molecules. In contrast, approaches to target cytokines and cytokine receptors using humanized blocking mAbs, soluble receptors and mutant cytokines are currently being investigated. As chemokines are involved in stimulating the migration of T_H2 cells and eosinophils into inflamed tissues, targeting these molecules and their receptors may be a useful strategy for the treatment of tissue inflammation in asthma and allergy. It is probable that a subset of chemokines are the key targets in asthma and are selective for eosinophils[108]. Selective eosinophil recruitment can be induced by chemokines that bind to chemokine (C-C motif) receptor 3 (CCR3) on eosinophils, such as eotaxin-1 (chemokine (C-C motif) ligand 1 (CCL11)), eotaxin-2 (CCL24), eotaxin-3 (CCL26), RANTES (CCL5) and MCP-4 (CCL13). Mice deficient in one of these chemokines have impaired eosinophil trafficking to the skin, airway and/or gut[109].

A key role for chemokine (C-X-C motif) receptor 2 (CXCR2) has been identified in asthma, chronic obstructive pulmonary disease and fibrotic pulmonary disorders. After CXCR2 inhibition, the angiogenesis and collagen deposition caused by lung injury is still observed, but the pulmonary damage induced by neutrophils, antigen or irritant-induced goblet cell hyperplasia is inhibited[110]. These features are common in inflammatory and fibrotic disorders of the lung. Clinical trials to evaluate small-molecule CXCR2 antagonists in chronic obstructive pulmonary disease, asthma and cystic fibrosis have been completed and have suggested that these molecules may be promising in the treatment of these conditions[110].

The prostaglandin and chemotactic receptors DP1 and CRT_H2 (also known as DP2) have key roles in the development and maintenance of allergy. Studies with DP1 and CRT_H2 antagonists have suggested that they could have a crucial role in modulating aspects of allergic diseases that are resistant to current therapies[111,112]. Prostaglandin D2 (PGD2) has a dominant role in mediating mast-cell–dependent activation of T_H2 lymphocytes, which is also mediated by CRT_H2 (ref. 112). PGD2 produced by mast cells might link the early and late phase allergic responses, and the antagonism of PGD2 is an attractive target for therapeutic intervention[111].

In people with asthma, a fraction of $CD4^+$ T cells express chemokine (C-X3-C motif) receptor 1 (CX3CR1), the receptor for CX3CL1. After allergen challenge, CX3CL1 expression is increased in the airway smooth muscle, lung endothelium and epithelium, and therefore this chemokine might be a target for immune modulation[113]. Consistent with this, wild-type mice treated with CX3CR1-blocking reagents and CXCR1-deficient mice show reduced inflammation in the lungs after allergen sensitization and challenge[113].

Biologicals in atopic dermatitis

A subgroup of patients with severe atopic dermatitis requires systemic immunomodulatory treatment. Clinical studies of omalizumab, efalizumab (a humanized antibody to CD11a), infliximab, adalimumab and etanercept (agents that target TNF-α signaling) and rituximab have been performed with varying results and safety profiles. Efalizumab inhibits T cell activation and thereby impairs the recruitment of T cells into the skin. A retrospective analysis of 11 of the patients in the efalizumab study showed that only 2 had a positive outcome. Nine patients stopped treatment because of a progression of atopic dermatitis or a lack of any clinical effect[114]. In the study, in the individuals who showed disease progression after treatment with efalizumab, it was not clear whether this progression was a result of side effects of the drug or was a natural progression of the disease.

TNF-α plays a part in inflammation and keratinocyte apoptosis, leading to eczema in atopic dermatitis[115]. Infliximab, a chimeric mAb to TNF-α, has been used to treat sporadic cases of atopic dermatitis and has also been tested in an open, prospective pilot study of nine patients with atopic dermatitis that was refractory to conventional therapy[97]. The results from the treated patients and the pilot study suggest that infliximab can be used as an additional therapeutic option for refractory severe atopic dermatitis. Etanercept, which is a soluble TNF receptor, has been used as a treatment option in two patients with severe, chronic atopic dermatitis and led to clinically significant improvements in skin symptoms. These preliminary results suggest that etanercept therapy may be beneficial for atopic dermatitis, particularly in chronic variants associated with lichenification, which is characterized by a thickening and hardening of the skin as a result of the disease[116]. Similar to asthma, safety concerns for treatments that target TNF-α may be a caveat in their use for atopic dermatitis.

Omalizumab has been used in selected patients suffering from both atopic eczema and allergic asthma or from atopic eczema alone[117]. Improvements seen after omalizumab treatment in these patients seem to be a result of more immune regulatory mechanisms rather than of simple neutralization of IgE by omalizumab. Recently, a randomized, placebo-controlled, double-blind pilot study of omalizumab was performed in 20 patients, which showed that the drug did not improve the clinical course in patients with chronic atopic dermatitis[118]; however, it did improve the results of skin prick tests and atopy patch tests.

Rituximab is a chimeric mAb to CD20 that efficiently eliminates circulating B cells. B cells that have class switched to produce IgE have long been suggested to have a role in atopic dermatitis because highly elevated concentrations of serum IgE are commonly observed in patients with generalized extrinsic atopic dermatitis. A pilot study with six patients was conducted in which rituximab was intravenously administered in two doses of 1,000 mg given 2 weeks apart[119]. All six patients showed an improvement of their skin symptoms within 4–8 weeks of the start of treatment, suggesting that rituximab may be a promising treatment option for patients with severe atopic dermatitis. However, these initial results have not been confirmed in two patients treated with rituximab who did not show amelioration of disease symptoms after treatment[120]. Therefore, larger randomized studies will be required to more rigorously test the effects and mechanisms of immune regulation induced by omalizumab and rituximab in the treatment of atopic dermatitis.

Conclusions and future perspectives

A better understanding of the molecular mechanisms involved in asthma and allergy is hoped to spur the development of new treatment modalities for these conditions. Currently, allergen SIT is the only available curative treatment for allergic diseases, as it can induce long-term allergen-specific immune tolerance using multiple mechanisms. Despite the benefits of this therapy for most treated individuals, not everyone improves; life-threatening side effects can occur,

recovery may not be permanent and the duration of the treatment is long. Therefore, new vaccines, as well as reliable biomarkers to select patients who may have a good clinical response, are crucial. Notably, allergen-SIT–based curative approaches may also be promising for the prevention of allergic disease, although the challenges accompanying the prevention approaches might differ to those for treatment. The major challenges for prevention include the requirement for very early intervention, safety problems for pediatric usage of vaccines and the lack of early biomarkers to predict who will develop allergies and to which particular allergen.

There is also a strong rationale for the development of biological immune response modifiers. The future of this area of research should be exciting, as advances in immunology and bioengineering are being applied, allowing for the optimized design of biologicals to improve the clinical efficacy and the feasibility of clinical grade production of these agents. Patient-specific treatments that depend on the development of new diagnostic biomarkers that can discriminate between distinct disease endotypes are also on the horizon. The analysis of large datasets of adults and children with severe allergic diseases and asthma is now beginning to allow for the identification of distinct disease phenotypes. It will be crucial to identify biomarkers and use systems biology to predict clinical responses for various subphenotypes of the diseases. This area requires more research for personalized therapy to become feasible because only a small proportion of patients with severe allergic diseases (for example, asthma) are likely to respond to a single biological. In particular, the combination of immune response modifiers with allergen SIT might provide a way for efficient immunomodulation of allergic diseases. Moreover, a greater understanding of the underlying disease mechanisms in allergic diseases could bring the possibility of cure in a larger patient population closer to reality.

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