Immune response modifiers in the treatment of asthma: A PRACTALL document of the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergy and Clinical Immunology

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Advances in molecular biology and immunology have led to a whole new algorithm for the treatment of autoimmune and allergic disorders from the traditional pharmacologic approach of drug therapy. These new therapeutic approaches, termed biologic immune response modifiers (IRMs), include therapeutic antibodies, small-molecule initiators of the innate immune system, and other biologic agents that target effector molecules, specific cells or cell-surface determinants, and cytoplasmic factors among others. More than 30 mAbs have been approved for various indications, especially for autoimmune disorders, organ transplantation, infectious diseases, and cancer. More than 250 antibodies are currently in clinical development.

The origins of these therapeutic antibodies date back to the early 1970s, with the use of antithymocyte globulin made in rabbits for the treatment of renal allograft rejection. Cesar Milstein, who had a longstanding interest in the mechanism of antibody diversity, together with Georges Kohler, fused myeloma cells with lymphocytes from animals immunized with a specific antigen to create hybridomas. These immortalized B cells, secreting a single type of specific antibody, led to the term *monoclonal antibodies*. In 1984, Neils Jerne, Cesar Milstein, and Georges Kohler shared the Noble Prize in medicine for the discovery of the principles of mAb production.[1]

Abbreviations used	
APC:	Antigen-presenting cell
CRTH2:	Chemoattractant receptor-homologous molecule expressed on T_H2 cells (also known as DP_2)
FDA:	US Food and Drug Administration
FENO:	Fraction of exhaled nitric oxide
IL-4R:	IL-4 receptor
IRM:	Immune response modifier
MPLA:	Monophosphoryl lipid A
PAMP:	Pathogen-associated molecular pattern
PPAR:	Peroxisome proliferator-activated receptor
Syk:	Spleen tyrosine kinase
TKI:	Tyrosine kinase inhibitor
TLR:	Toll-like receptor
VLA-4:	Very late antigen 4

In 1986, OKT3 (muromonab), a CD3-specific murine mAb was the first US Food and Drug Administration (FDA)–approved mAb for the treatment of acute transplant rejection. The initial therapeutic mAbs were murine proteins. To make the antibodies less antigenic and thus less likely to elicit adverse reactions, chimeric mAbs were created by combining the murine antibody variable region that contains the antigen-binding region with the constant region of a human immunoglobulin molecule (Fig 1). Today, most
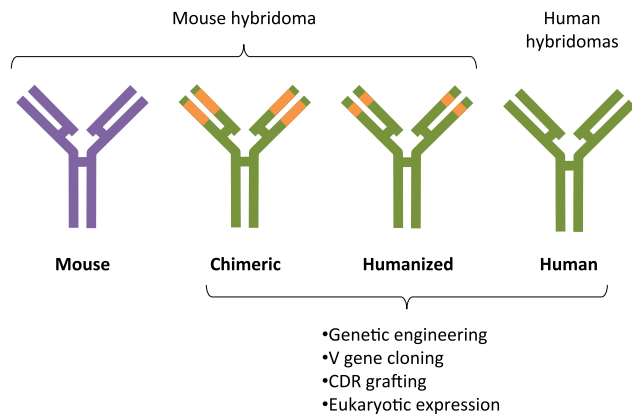
FIG 1. The transition of mAbs from all murine protein to chimeric and humanized mAbs in which part of the antigen-binding region is of the mouse (*orange areas* in the variable regions of the heavy and light chains) to a fully human mAb. *ADCC,* Antibody-dependent cell-mediated cytotoxicity. Reproduced with permission from Beck et al.[128]

therapeutic mAbs are either humanized, incorporating only the hypervariable region of the murine mAb, or totally human mAbs made through genetic engineering. Table I shows the naming nomenclature for mAbs. A new nomenclature scheme by the United States Adopted Name Council supersedes previous schemes and adds naming protocols for posttranslational modifications. This new nomenclature scheme will not affect the names of previous mAbs. At the end of the article, we will discuss some of the newer innovative approaches and designs for therapeutic antibodies.

Because an extensive range of immunomodulators are being developed for asthma, it has been less obvious whether these are going to be effective in most or all patients with asthma or whether they will be therapeutic only in subgroups of asthmatic patients. Classically, asthma is defined as "a common chronic disorder of the airways that is complex and characterised by variable and recurring symptoms, airflow obstruction, bronchial hyperresponsiveness, and an underlying inflammation." However, the complexity of asthma is vast, with different patients having differences in natural history, severity, comorbidities, and response to different treatments.[2] In fact, recent studies have proposed that patients with asthma express substantially different observable characteristics, such as phenotypes and endotypes of disease. Specifically, asthma phenotypes have been proposed to involve clinical characteristics, physiologic measures, inflammatory parameters, and laboratory data, as well as the response to different treatments.[3,4] When the phenotypic characteristics of large cohorts of asthmatic patients are studied, diverse clusters of disease can be observed.[5,6] Overall, these findings therefore argue that asthma is not a single disease but rather a syndrome of several subtypes with overall similar symptomatology among all patients with the diagnosis but with different fundamental mechanisms and pathobiological processes causing disease in different patients. Because the term *phenotype* does not incorporate mechanisms of the observed phenotypic characteristics, it was recently proposed that the term *endotype* should be used to define a distinct subtype of asthma with a distinct mechanism.[7,8] Endotype is thus a different classification from phenotype and describes distinct disease entities with distinct immune and perhaps inflammatory pathophysiology. Thus the asthma syndrome is comprised of several endotypes, with most being mechanistically poorly understood. However, when medicines are developed to target very

specific disease processes, as is the case with most novel immunomodulating therapies, it is likely that each medication will function only in specific asthma endotypes. As such treatments are developed, it is therefore crucial that patients with very distinct phenotypic characteristics, which are likely to encompass distinct asthma endotypes, are studied. Because IRMs are being developed for the treatment of asthma, linking the target molecule, cell, or both of each treatment to the asthma endotype is important for deciding the inclusion criteria in clinical studies and decisions for specific treatment selections.

In this report we will review the potential IRMs that target the immune and inflammatory pathways in asthmatic patients and discuss IRMs that might be potentially of interest in certain asthma endotypes. We will start our discussion with the most successful of the IRMs in asthmatic patients that target the IgE molecule. In addition, we will discuss other IRMs that target other components of the allergic pathway, such as cytokines, cell-surface receptors, and signaling molecules.

Our working definitions of an IRM is any substance or molecule that binds to or interferes with the binding to a receptor or a ligand on or in cells that are integral to a specific immune pathway, resulting in deletion, amplification, diminution, or functional shift of such cells.

IRMs THAT NEUTRALIZE EFFECTOR MOLECULES

Omalizumab (Xolair; Genentech, South San Francisco, Calif) is a 95% humanized mAb that forms soluble immune complexes with free IgE at the same site, which normally binds the high-affinity IgE receptor FcεRI, thus preventing cross-linking of FcεRI and subsequent basophil and mast cell activation. Omalizumab can reduce serum free IgE levels by 99% within 2 hours of administration and can reduce nasal allergen challenge responses within 2 weeks of therapy initiation.[9] Omalizumab also decreases the expression of FcεRI on basophils, dendritic cells, and monocytes within 7 days.[9,10] In asthmatic patients omalizumab reduced serum, tissue, and sputum eosinophilia and numbers of cells positive for FcεRI. After 16 weeks of treatment, omalizumab was shown to decrease B-cell numbers and $CD3^+$, $CD4^+$, and $CD8^+$ cell numbers in lung tissue.[11]

In patients receiving inhaled corticosteroids alone or in combination with other agents, the addition of omalizumab has been shown to reduce the number of exacerbations (by about 50%) and improve symptom scores, the need for inhaled corticosteroids, the use of rescue medication, asthma-related quality of life, and pulmonary functions (modestly). These effects appear independent of duration of treatment, age of the patient, and severity of asthma.[12] The reasons for omalizumab being ineffective for some (approximately 40%) patients are unknown. Improvements correlate with IgE level reductions, but free IgE levels in nonresponders are similar to those found in responders.[13] Possible reasons include (1) the relationship between free IgE levels and FcεRI expression, (2) the ratio of specific IgE to total IgE, and (3) intrinsic cellular sensitivity.[14] In a study by Bousquet et al,[15] the global evaluation of the treatment effectiveness score was used to assess the response to omalizumab at 16 weeks of treatment. An excellent or good global evaluation of treatment effectiveness score was an effective predictor (83%) of continued persistent response to omalizumab at week 32 of the study. Whether omalizumab can be stopped with sustained clinical efficacy is unclear and might depend on the duration of treatment.[13,16] Omalizumab has a

TABLE I. Monoclonal antibody nomenclature

Prefix		Target inflix		Source inflix	Suffix
Varies, should be euphonious	-vir(r) -/-v-	Viral	-o-	Mouse	-mab
	-ba(c)-/-b-	Bacterial	-a-	Rat	-pab
	-le(s)-	Infectious lesions	-e-	Hamster	
	-ci(r)-/-c-	Cardiovascular	-i-	Primate	
	-ki-/-k-	Interleukin as target	-xi-	Chimeric	
	-tu(m)-/-t-	Tumor	-zu-	Humanized	
	-li(m)-/-l-	Immune	-u-	Human	
	-ne-/-n-	Nervous system	-axo-	Rat/murine hybrid	
	-tox(a)-	Toxin as target			
	-fu(ng)-/-f-	Fungal			

If the antibody is conjugated to a product, such as a radiolabel or toxin (-tox-), this conjugate is identified by using a separate second word or chemical designation.
Examples: Adalimumab: Ada + lim (immune) + u (human) + mab (mAb) - mab to TNF-α; Efalizumab: Efa + Li(m) (immune) + zu (humanized) + mab - mAb to CD11a.
Adapted from the United States Adopted Names Council (USANC) at USAN@ama-assn.org. The USANC is trisponsored by the American Medical Association, the United States Pharmacopeia Convention, and the American Pharmacists Association. In addition, the FDA cooperates with and is represented on the USANC. The USANC aims for global standardization and unification of drug nomenclature and related rules to ensure that drug information is communicated accurately and unambiguously. The USANC works closely with the International Nonproprietary Name Program of the World Health Organization and various national nomenclature groups.
-mab, Monoclonal antibody; *-pab*, polyclonal mixture of recombinant mAbs.

relatively good safety profile, but concerns have been raised about cancer, cardiovascular events, and anaphylaxis, which occurs in about 0.1% to 0.2% of patients.[17,18]

Pretreatment of patients with asthma and rhinitis with omalizumab has been shown to add efficacy and safety to allergen-specific immunotherapy and allowed more patients to reach the maintenance dose. However, it unknown how long you need to treat with both and whether you can stop the omalizumab after reaching maintenance immunotherapy.[19,20]

Omalizumab is also being investigated in patients with eosinophilic esophagitis, allergic bronchopulmonary aspergillosis, hyper-IgE syndrome, idiopathic anaphylaxis, food allergy, and chronic urticaria.[21,22]

IRMs THAT AFFECT THE T$_H$2 PATHWAY

The T$_H$2 pathway was first described in mice and human subjects well over 20 years ago. Not long after, speculation arose that the T$_H$2 pathway was important in asthmatic patients based on murine studies that suggested that IL-13, in particular, was a critical molecule in the development of allergic inflammation and bronchial hyperresponsiveness. However, despite this initial enthusiasm, studies of molecules that modulated this T$_H$2 pathway in human subjects were limited and slow to evolve. In fact, results of early studies with both a soluble IL-4 receptor (IL-4R) and an mAb to IL-5 were negative, further dampening enthusiasm. In recent years, the concept of asthma phenotyping, or ideally endotyping, has re-energized the approach to modulating the T$_H$2 process. Approximately 50% of patients with mild untreated asthma have an airway T$_H$2 signature.[23] Thus focusing T$_H$2-modulating therapies on this subgroup would seem important.

INTERRUPTION OF THE IL-4, IL-13, AND IL-4Rα PATHWAYS

The first approach was to block the effects of IL-4 with a soluble IL-4R. A small 25-subject study used a corticosteroid withdrawal design in patients with mild-to-moderate asthma.[24] A single nebulized dose of placebo was compared with 2 doses of soluble IL-4R over a 2-week period after the discontinuation of inhaled corticosteroids. The highest dose was effective in limiting the decrease in lung function while preventing loss of symptom control or exacerbations. The level of exhaled nitric oxide also decreased with the highest dose, but no effect was seen on systemic markers of inflammation. A larger study of 62 subjects showed some effect at the highest dose (3.0 mg), but the clinical changes were small.[25] In addition, anti–IL-4 mAb had no significant effects on asthma parameters, suggesting that therapies aimed at IL-4 alone might not be sufficient to invoke positive therapeutic effects.

Although the early anti–IL-4 approaches only targeted IL-4, murine studies suggested that IL-13 would be a more important target for allergic inflammation. Thus pitrakinra, a recombinant IL-4 molecule mutated at 2 critical amino acid sites (arginine to aspartic acid at 121 and tyrosine to aspartic acid at 124), was developed. Pitrakinra bound competitively but nonfunctionally to cell membrane IL-4Rα, the signaling component of the heterodimeric receptor complex for both IL-4 and IL-13, therefore acting as an antagonist of these 2 cytokines. Initial studies were done with a subcutaneous injection of drug, which, likely because of the short half-life of the drug, were not very efficacious. However, a second study delivered the mutant IL-4/pitrakinra (or placebo) through the nebulized route to 32 patients with mild allergy for 4 weeks before comparing the posttreatment response to inhaled allergen challenge with the baseline response.[26] Pitrakinra produced a modest and marginally significant decrease in the immediate response and an approximately 50% decrease in the late asthmatic response compared with baseline ($P < .0001$), whereas placebo had no effect on either response. Similar to the earlier study of nebulized IL-4R, fraction of exhaled nitric oxide (F$_{ENO}$) values were again significantly decreased before allergen challenge compared with those after placebo, whereas there was a trend toward reduction in F$_{ENO}$ values after challenge. In an 8-week study, pitrakinra was studied in more than 500 patients with moderate-to-severe asthma receiving combination therapy.[27] For the first 4 weeks, the combination therapy was held constant. Beginning at the fifth week, the long-acting β-agonist was first tapered and then followed by sequential halving of the inhaled corticosteroid dose, each at 2-week intervals. The primary end point was time to the first exacerbation. Although there was no treatment effect in the overall

population, in a prespecified subgroup analysis of subjects with more than 350 eosinophils/mm in the peripheral blood, there was a significant reduction in time to first exacerbation, as well as the severity of the exacerbation, with the greatest effect seen with the highest dose. In addition to time to first exacerbation, symptom scores did not worsen in the pitrakinra-treated groups, with the greatest efficacy seen at the highest dose.

In a 12-week, phase 2, randomized, placebo-controlled study of a humanized mAb to IL-4Rα (AMG 317), which prevents binding of IL-4 and IL-13 to their common receptor component, patients received weekly subcutaneous injections of placebo or varying doses (75-300 mg) in patients with moderately symptomatic asthma.[27] Despite promising efficacy in allergen challenge models, this particular humanized mAb to IL-4Rα did not improve its 2 primary outcomes: the Asthma Control Questionnaire score and FEV$_1$. Of note, despite the lack of efficacy, serum IgE levels were decreased by approximately 50% with the high dose. In an attempt to determine whether a subgroup might respond better to AMG 317, the subgroup with the worst Asthma Control Questionnaire score appeared to have a modest response to the highest dose of the drug. Despite this small signal, this particular antibody is not being taken forward in the treatment of asthma.

Using allergen challenge to investigate 2 neutralizing mAbs against IL-13 (which recognized different epitopes), Gauvreau et al[28] saw a significant inhibition of both the early- and late-phase response with one but not the other. No effect on sputum eosinophil numbers or airway hyperresponsiveness was observed. In a recent study of an mAb directed against IL-13 (lebrikizumab), Corren et al[29] reported a modest improvement of 5.5% in predicted prebronchodilator FEV$_1$ (the primary outcome measure) in patients with moderate-to-severe asthma, including those taking long-acting β-agonists.[29] Dividing the subjects into those with blood eosinophils, IgE, or both did not improve the identification of responders. However, when serum periostin levels was used to divide the population, those in the upper 50th percentile had an 8.2% improvement in FEV$_1$ compared with the low-periostin group, who had no improvement in predicted FEV$_1$. Periostin had previously been identified as a molecule highly expressed by epithelial cells (and likely other cells) in response to IL-13 and therefore was suggested to be a serum T$_H$2 biomarker.[23] Interestingly, F$_{ENO}$ values were similarly predictive of response, and in fact, levels decreased with anti–IL-13 therapy. There was no effect on symptoms, but there was a 60% reduction in exacerbations in a T$_H$2-high group defined according to total IgE levels and peripheral blood eosinophil numbers. Thus a patient with an asthma endotype with increased serum periostin levels appears to be a good candidate for anti–IL-13 therapy.

ANTI–IL-5

The close association between eosinophils and asthma has been appreciated for many decades.[30] In the 1980s, a series of studies used bronchoscopy to demonstrate that eosinophilic airway inflammation was a feature of even mild asthma, and this was one of the justifications for the widespread introduction of inhaled steroids for patients with all severities of asthma.[31] The idea that eosinophils were one of the major effector cells in asthmatic patients was underpinned by the paradigm that asthma was driven by T$_H$2-mediated inflammation because there is a close relationship between T$_H$2-derived cytokines and eosinophil recruitment and function. However, the importance of eosinophils in asthmatic

patients was called into question by early studies of 2 antibodies that neutralized IL-5, an obligate growth factor for eosinophil expansion. Leckie et al,[32] in an allergen challenge study, found that the GlaxoSmithKline (Research Triangle Park, NC) antibody mepolizumab was very effective at reducing sputum and blood eosinophil numbers but had no effect on prechallenge airway hyperresponsiveness or the reduction in FEV$_1$ during the early and late response. A subsequent clinical trial of mepolizumab in 362 patients with moderate asthma found no effect on standard asthma outcomes, including lung function, symptoms, and β$_2$-agonist use. There was a trend toward a reduction in exacerbations, but the study was underpowered to investigate this outcome.[33]

Kips et al[34] undertook a small study to determine the safety of another anti–IL-5 antibody (SCH55700, reslizumab) developed by Schering-Plough (Deerfield, Ill) in patients with severe asthma. They found a modest improvement in FEV$_1$ but no effect on other clinical parameters. There were 2 important caveats to these clinical studies. First, they did not target patients with eosinophilic disease. This is important because, as pointed out above, not all patients with asthma have evidence of excess eosinophils.[35,36] Second, Flood-Page et al[37] demonstrated in a study of patients with mild asthma (in which they also found no effect on standard asthma outcomes or airway hyperresponsiveness) that although mepolizumab caused a marked reduction in blood and sputum eosinophil numbers, it only reduced tissue eosinophil numbers by approximately 50%. Even this might be an overestimate because some eosinophils in bronchial biopsy specimens would be within the blood vessels.

However, the most important weakness of all the studies discussed above was that they might have targeted the wrong outcome measure. There is actually very limited evidence that eosinophils are important in driving variable airflow obstruction, the classical physiologic measure that defines asthma.[38] Because airway hyperresponsiveness, day-to-day symptoms, and bronchodilator use are closely linked to this physiologic abnormality, it means that studies that use these measurements as outcome measures of antieosinophil treatment will not be successful in showing any benefit. Asthma, particularly in its more severe forms, consists of 5 pathophysiologic abnormalities, which, although interrelated, are relatively distinct: airway hyperresponsiveness, bronchitis (eosinophilic or neutropenic airway inflammation), cough reflex hypersensitivity, lung damage as reflected in bronchiectasis and irreversible airflow obstruction, and extrapulmonary factors.[39] Eosinophils are most closely linked to severe exacerbations.[40]

Two recent, small, single-center studies have addressed the deficiencies identified above by investigating mepolizumab in patients with eosinophilic asthma by using severe exacerbations as the primary outcome. Nair et al[41] investigated 18 patients with highly eosinophilic, oral corticosteroid–dependent asthma by using a steroid-reduction design and found a marked difference in the rate of exacerbations and the ability to reduce the dose of oral steroids between the active and placebo groups. As pointed out by Wenzel in a commentary on the article,[42] the patients in this study represented a relatively small subgroup of patients with asthma-like airway disease, perhaps more akin to patients with hypereosinophilic disease, in whom mepolizumab had been shown to be beneficial,[43] who might not necessarily have airway hyperresponsiveness or variable airflow obstruction. The extent to which this study can be generalized to the wider asthmatic population can be questioned.

In contrast, the study by Haldar et al[44] recruited 61 subjects with eosinophilic asthma from a difficult asthma clinic population in which at least 25% of the patients attending the clinic would meet the recruitment criteria of a history of severe exacerbations and a sputum eosinophilia of greater than 3% despite high dose inhaled corticosteroids. They found a significant reduction of approximately 40% in the number of severe exacerbations over the 12-month course of the study. As in previous studies, there was a marked, although not complete, reduction in sputum eosinophil but not tissue eosinophil numbers. The greater the reduction in sputum eosinophil numbers, the lower the number of exacerbations. Strikingly, there was no difference between the active and placebo groups in the standard asthma markers of FEV_1, airway hyperresponsiveness, day-to-day symptoms, or β_2-agonist use, although there was an overall improvement in quality of life, thus emphasizing the distinction between the pathophysiologic pathways that lead to variable airflow obstruction (presumably secondary to smooth muscle dysfunction) and those related to eosinophilic inflammation. Interestingly, there was an improvement in the computed tomographic scan appearances, suggesting an effect on airway remodeling. This effect was also suggested by a reduction in basement membrane thickening in the active group shown by Flood-Page et al[45] in their studies with mepolizumab.

These studies have encouraged GlaxoSmithKline to undertake a multicenter study of mepolizumab in patients with eosinophilic asthma. In addition, Cephalon (Frazer, Pa) have obtained the rights to SCH55700 (as reslizumab) and are embarking on a program of studies investigating the antibody in eosinophilic asthma by using severe exacerbation as one of the outcome measures. They have already reported a 3-month study in patients with moderate eosinophilic asthma, which again demonstrated about a 50% reduction in exacerbations and, interestingly, a modest improvement in FEV_1.[46] Lastly, MedImmune (Gaithersburg, Md) are taking forward an mAb (MEDI-563) that binds to the IL-5 receptor, preventing IL-5 binding and also inducing eosinophil cell death.[47] This is particularly exciting because it offers a better chance of reducing tissue eosinophil numbers.

There is therefore increasing evidence that antieosinophil strategies will at least play an important role in preventing severe exacerbation of asthma in which the asthma endotype includes an important role for eosinophils. Current evidence suggests that these treatments are safe and well tolerated. The full extent of the role of eosinophils in asthmatic patients should become clear within the next couple of years.

MODULATION OF THE CHEMOATTRACTANT RECEPTOR-HOMOLOGOUS MOLECULE EXPRESSED ON T$_H$2 CELLS RECEPTOR

Prostaglandin D_2 is a major lipid mediator produced (outside the brain) mainly by mast cells. It is present in increased amounts after allergen challenge, although concentrations in sputum and bronchoalveolar lavage fluid were not markedly increased in patients with clinical disease.[48,49] There are 3 receptors for prostaglandin D_2. The DP1 receptor is expressed on airway smooth muscle, vascular tissue, and T cells and mediates bronchodilation and immune modulation. The thromboxane receptor is expressed on airway smooth muscle and mediates bronchoconstriction. The chemoattractant receptor-homologous molecule expressed on T$_H$2 cells (CRTH2) is a chemoattractant receptor expressed on

a (small) subset of T$_H$2 cells, eosinophils, and basophils. Antagonists of DP1 and the thromboxane receptor have not been effective in asthmatic patients.[50] However, it appears relatively straightforward to make potent, orally available antagonists of CRTH2, and a number are in clinical development.[51] Animal studies of asthma models involving CRTH2 have had mixed results. For example, gene deletion of CRTH2 demonstrated increased eosinophilic inflammation after short-term exposure to allergen but decreased after long-term exposure.[52] A more consistent effect has been seen in murine models of atopic dermatitis with a reduction in inflammation with gene deletion.[53]

Despite the interest in this receptor as a target for asthma and treatment of 400 patients with anti-CRTH2 antagonists, only 1 study in asthma has been reported in the literature. Barnes et al,[54] in a 1-month study of steroid-free adult subjects with moderate persistent asthma, administered 200 mg of oral OC000549 twice daily, a potent, orally available CRTH2 antagonist that has previously been shown to reduce the late response after allergen challenge. Change in FEV_1 was the primary outcome measure. There was a modest (7%) improvement in FEV_1, which was significant in the per-protocol population but not the full population. Quality-of-life and nighttime symptoms scores also improved. There was also a trend toward a reduction in sputum eosinophil numbers. Other selective CRTH2 antagonists have been developed for the treatment of respiratory disease but have not yet been studied in patients with asthma.[55]

ANTAGONISTS OF ADHESION AND CHEMOKINE PATHWAYS CONTROLLING EOSINOPHIL AND T$_H$2 LYMPHOCYTE MIGRATION

Leukocyte migration into tissue is a fundamental part of the immune response, which is controlled by a combination of growth factors that produce the cells in the bone marrow, and adhesion and chemotactic pathways (in particular chemokines and their receptors) that control egress into the vasculature and migration into tissue through postcapillary venules. There are both homeostatic pathways that control immune surveillance in health, and inflammatory pathways that are switched on in an organ-specific manner when tissue injury occurs, which is where the opportunity for therapeutic intervention lies. There is a degree of specificity in the pathways controlling migration both in terms of the leukocyte and organ involved, and this encouraged the hope that it might be possible to antagonize leukocyte migration without causing intolerable immune suppression. Eosinophil adhesion to the endothelium is preferentially (compared with neutrophils) mediated by very late antigen 4 (VLA-4)–vascular cell adhesion molecule 1 and P-selectin glycoprotein ligand 1. This is due to a relative lack of expression of VLA-4 by neutrophils and greater avidity of binding of eosinophils to P-selectin.[56] However, a synthetic panselectin antagonist, bimosiamose, had relatively low potency and was a more effective antagonist of neutrophil than eosinophil adhesion.[57,58] Beeh et al[59] evaluated the effect of inhaled bimosiamose (TBC1269) on allergen-induced late asthmatic response in 12 adult male asthmatic patients. Bimosiamose significantly attenuated the maximum late asthmatic response by 50% compared with placebo. There was no effect on the early asthmatic response, postallergen airway hyperresponsiveness, F$_{ENO}$ values, or peripheral blood cell numbers.

Antibodies against P-selectin have not been tried in asthmatic patients. Vascular cell adhesion molecule 1 gene deletion is embryonically lethal in mice, and as noted below, antibodies against VLA-4 have led to major side effects (as have antibodies against lymphocyte function-associated antigen 1, a more ubiquitously functional adhesion receptor). Antagonists of chemokines or chemokine receptors have been equally slow to show promise as therapeutic agents. Chemokines are a large family of about fifty 8- to 10-kDa cytokine chemoattractants divided into 4 families based on their structure. They bind relatively specifically to about 28 seven-transmembrane, G protein–linked receptors.[60] Antagonists of CCR3, the major chemokine receptor involved in eosinophil migration, have great promise theoretically, and several potent antagonists have been developed. However, few clinical trials have been reported. An antisense oligonucleotide against CCR3 in combination with an antisense oligonucleotide against the common β-chain of the IL-5–GM-CSF–IL-3 family attenuated the late response to allergen challenge.[61] CCR4, which binds CCL17 and CCL22, has attracted a lot of attention as a relatively T_H2-specific chemokine receptor that might play a role in the recruitment of T_H2 cells into the lung.[62] CCR8 is a more specific T_H2-associated receptor but is also expressed on regulatory T cells.[63] Tian et al[64] showed in a murine model of asthma that a human chemokine-like factor 1 C-terminal peptide (C19), which blocks the function of the chemokine receptor CCR4, reduced airway eosinophilia and airway hyperresponsiveness. There are no reports of the antagonists of any of these receptors in asthmatic patients.

Generally, there are not enough clinical data to determine whether antagonists of eosinophil- and T_H2 lymphocyte–associated adhesion and chemokine pathways are likely to be fruitful. CXCR1 and CXCR2 are the major chemokine receptors controlling neutrophil migration, in part through binding of CXCL-8 (IL-8). Orally available antagonists of these receptors are in clinical trials but are not yet reported in detail, and the endotype of neutrophilic asthma is not sufficiently well understood to clearly see where these antagonists would be optimally used.

IL-9 AND MORE RECENTLY DESCRIBED T_H2-ASSOCIATED CYTOKINES

IL-9 is a pleiotropic cytokine originally described in 1990, which maps to the long arm of chromosome 5. Initially, it was thought to be a T_H2-associated cytokine, but relatively recently, a new subset of T cells have been described that produce significant amounts of IL-9. However IL-9 is also produced by T_H17 cells, regulatory T cells, eosinophils, and mast cells.[65] The IL-9 receptor is a heterodimer consisting of a specific α chain and the common cytokine γ chain. The receptor is expressed on mast cells, in which it promotes growth and proliferation; T cells; antigen-presenting cells; epithelial cells; and goblet cells. T_H9 cells are produced in vitro by the combined effect of TGF-β, IL-4, and IL-2 and are dependent on the transcription factors PU.1 and interferon regulatory factor 4. T_H9 cells produce IL-10 in mice but not in human subjects. Antagonism of IL-9, either by means of gene deletion or antibody, attenuated or abolished lung function and inflammation-related changes in an ovalbumin murine asthma model.[66-68] Overexpression of IL-9 resulted in goblet cell hyperplasia, eosinophilia, and increased airways reactivity,[69] possibly by inducing IL-13 expression in bronchial epithelial cells.[70] There are relatively few data about IL-9 expression in asthmatic patients, although it was shown by means of in situ hybridization to be increased in bronchial biopsy specimens.[71] No genetic link has emerged. Clinical trials of antagonists of IL-9 appear limited to a program by MedImmune involving an mAb against IL-9: MEDI-528. They have reported 2 small dose-ranging studies in patients with mild-to-moderate asthma that demonstrated no safety concerns and suggestions of clinical benefit.[72] Efficacy studies are awaited.

IL-33 is a member of the IL-1 family of cytokines that binds to ST2, a heterodimeric receptor expressed on mast cells and epithelial cells. IL-33 is produced by a wide range of cells, including alveolar macrophages and epithelial cells in the lung. As well as promoting the development of T_H2 cells, IL-33 also appears pivotal in the production of the newly described IL-5– and IL-13–producing innate lymphoid cells and might therefore have a broad function in promoting eosinophilic inflammation. As yet, no clinical studies of antagonists have been reported.[73]

Thymic stromal lymphopoietin is an epithelium-derived cytokine that can promote T_H2 patterns of inflammation by promoting the generation of T_H2 cells through dendritic cell interactions and by activating mast cells. Blockade in the murine asthma model reduces inflammation, and overexpression promotes it. Expression is increased in asthmatic patients, particularly in those with more severe disease, and appears to correlate with the expression of other markers of T_H2 inflammation.[74] No studies of antagonists have yet been reported.

IL-25 was first described in expression libraries from T_H2 cells. Overexpression in airway epithelial cells leads to eosinophilic airway inflammation, and gene deletion or neutralizing antibody resulted in reduced inflammation and airway hyperresponsiveness in the murine asthma model.[75] Its effects could be mediated by promoting thymic stromal lymphopoietin/dendritic cell–activated T_H2 cells or through the innate lymphoid pathway. It is expressed in the bronchial mucosa in asthmatic patients.[76] No antagonists have yet been reported.

In summary, as noted above, a number of approaches are being taken to inhibit the T_H2 cytokine pathway. Eosinophilic inflammation, which is the hallmark of this pathway, is not found in all asthmatic patients, and thus treatment will need to be targeted by using biomarkers, such as sputum or blood eosinophils, exhaled nitric oxide, or periostin. There is increasing evidence that the physiologic hallmark of asthma, variable airflow obstruction, is only indirectly related to T_H2-mediated inflammation and that the major clinical readout of this pattern of inflammation is severe exacerbations. Antagonists of the T_H2 pathway might therefore be more effective against this outcome measure than classical measures of asthma improvement.

INHIBITION OF T CELLS AND THEIR CORECEPTORS

As discussed above, the T_H2 pathway is important in contributing to the airway inflammation seen in asthmatic patients. In patients with severe asthma, increased numbers of activated T cells and increased levels of IL-2 and soluble IL-2 receptor α chain can be found in the airways. Daclizumab, a humanized IgG$_1$ mAb directed against the IL-2 receptor α chain (CD25) subunit of the high-affinity IL-2 receptor, inhibits IL-2 binding and its biological activity. Daclizumab is approved for use in renal allograft rejection. In a phase 2 trial Busse et al[77] and, more recently, Nelson et al[78] reported that daclizumab improved asthma control in a subset of patients with refractory asthma. Sornasse et al[79] reported

that daclizumab can inhibit *in vitro* all the major T_H2 cytokines, including IL-4, IL-5, and IL-13. In a randomized placebo-controlled trial of adults with moderate-to-severe asthma, daclizumab improved FEV_1, reduced daytime asthma symptoms and the use of short-acting albuterol rescue, and increased the time to first exacerbatio.[80] Adverse events were similar between the treatment and placebo groups. However, 5 patients (of 88 treated with daclizumab) reported serious adverse events, including 1 patient with anaphylaxis. Daclizumab might work by blocking IL-2–induced T-cell proliferation and reducing the ability of these T cells to produce proinflammatory cytokines. At the present time, further development of daclizumab as asthma therapy is not planned.

T cells require at least 2 signals to become activated. The first signal is antigen specific and delivered through the engagement of the T-cell receptor with the MHC-peptide complex on the antigen-presenting cell (APC). The second is delivered through binding of a costimulatory receptor on T cells to its ligand on the APC. A key costimulatory signal is provided by the interaction of CD28 on T cells with CD80 (B7-1) or CD86 (B7-2) on APCs.[81] With engagement of the T-cell receptor and the CD28 costimulatory receptor, T cells proliferate and produce cytokines that activate other inflammatory cells, such as macrophages and dendritic cells. Another costimulatory molecule expressed on T cells, such as cytotoxic T lymphocyte–associated antigen 4, is expressed later during T-cell activation and is the high-avidity receptor for both CD80 and CD86. This interaction between cytotoxic T lymphocyte–associated antigen 4 on the T cell and its corresponding ligand on APCs provides a negative signal to the T cell to downregulate its immune reactivity. Activated T-cell interaction with APCs can be inhibited by blocking the T-cell receptor OX40. Monoclonal antibodies against OX40 ligand have been developed, and both phase 1 and proof-of-concept studies have been done in asthmatic patients.

IRMs THAT AFFECT SIGNALING PATHWAYS

The advantage of mAbs is their ability to bind to receptors expressed on the cell surface or bind and neutralize soluble mediators released from effector cells. However, mAbs cannot pass through the cell membrane and act on signaling pathways or transcription factors. However, small-molecule inhibitors can pass into the cytoplasm and can target various molecules that might interrupt the cellular machinery. Engaging certain receptors on the cell surface triggers a cascade of activation of various signaling molecules that proceeds in an orderly fashion, culminating in the activation of nuclear factor of activated T cells or nuclear factor κB, which then results in the differentiation of lymphocytes and the secretion of various cytokines. Inhibiting one of the molecules along such a cascade would be expected to result in inhibition of lymphocyte differentiation and cytokine secretion and might accelerate the induction of apoptosis. Several signaling pathway IRMs have been used in patients with atopic diseases.

Calcineurin inhibitors

Some of the molecules along the signaling cascade serve as a convergence point for several proximal signaling molecules. Calcineurin is one such molecule.

Cyclosporine is the prototype of calcineurin inhibitors, but tacrolimus and pimecrolimus are the ones most commonly used in patients with atopic disease because of their availability as topical agents. Both are approved by the FDA as second-line agents for the short-term treatment of moderate-to-severe atopic dermatitis in patients who are older than 2 years and immunocompetent. A subset of patients have exacerbation of their asthma on ingestion of aspirin or related products that is thought to be mediated by overproduction of cysteinyl leukotrienes. Tacrolimus, a macrolide-derived immunosuppressant, inhibits the release of cysteinyl leukotrienes. Kawano et al[82] reported that tacrolimus inhibited aspirin-induced bronchoconstriction and reduced urinary leukotriene E_4 excretion in patients with aspirin-induced asthma. However, Stevenson et al[83] did not show a similar effect of tacrolimus to prevent aspirin-induced asthmatic reactions. In a recent study by Virtanen et al,[84] long-term treatment of moderate-to-severe atopic dermatitis with topical tacrolimus decreased respiratory symptoms and bronchial hyperresponsiveness, but controlled trials are needed.

Tyrosine kinase inhibitors

Phosphorylation of various tyrosine kinases is an essential step in the activation and proliferation of lymphocytes. Tyrosine kinases are usually associated with the cytoplasmic domain of various receptors that have an extracellular domain as well. Inhibition of tyrosine kinase activity can therefore be accomplished by antibodies directed against an extracellular domain of a receptor that activates a tyrosine kinase (eg, antibodies directed against vascular endothelial growth factor, epidermal growth factor receptor, or human epidermal growth factor receptor 2). A more direct approach is the use of small molecules that inhibit (selectively or semiselectively) distinct molecules that are not expressed on the cell surface (referred to as tyrosine kinase inhibitors [TKIs]). Imatinib is the prototype of a TKI. It was initially introduced for its ability to bind Bcr-Abl, inducing apoptosis in patients with chronic myelogenous leukemia. It was later found that imatinib can also bind c-Kit and platelet-derived growth factor receptor. A subset of patients with systemic mastocytosis who have wild-type c-Kit respond favorably to imatinib. Patients with systemic mastocytosis who have the D816V kit mutation do not respond to imatinib because this mutation interferes with binding of imatinib to c-Kit.[85] A subset of patients with idiopathic hypereosinophilic syndrome (those with mutations in *FIP1L1*/*PDGFRA*) have a good response to imatinib.[86] Newer TKIs are available and might obviate the restrictions imposed by the mutations mentioned above on therapeutic responsiveness.

Other kinase inhibitors

Of the other kinases the modulation of which might be important in atopic diseases, spleen tyrosine kinase (Syk) has been examined most closely. Syk is expressed in most hematopoietic cells and is involved in signaling through the immunoreceptor tyrosine-based activation motif in the cytoplasmic domains of Fcγ and Fcε receptors; Syk is also involved in integrin signaling. One Syk inhibitor (R112) was found to reduce global symptom complex scores significantly as early as 45 minutes after dosing (and lasting at least up to 20 hours) in patients with allergic rhinitis.[87] However, comparison of R112 with beclomethasone and placebo in a 7-day trial of allergic rhinitis found it to be

identical to placebo. An inhaled Syk inhibitor has undergone early clinical studies in asthmatic patients.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR AGONISTS

Peroxisome proliferator-activated receptors (PPARs), particularly α and γ, can downregulate GATA-3, a key transcription factor for T_H2 cytokines, and inhibit activator protein 1, signal transducer and activator of transcription 1, nuclear factor κB, and nuclear factor of activated T cells. PPAR agonists also inhibit the chemotaxis of eosinophils and antibody-dependent cellular cytotoxicity.[88-90] Agonists of PPAR-γ include the oral hypoglycemic agents thiazolidinediones, rosiglitazone, and pioglitazone. The bronchodilatory effects of rosiglitazone were demonstrated in 46 patients with mild-to-moderate asthma after 4 weeks, but no significant improvements in asthma symptoms were shown.[91] Results of an open-label pilot study in 14 steroid-naive asthmatic patients given rosiglitazone demonstrated trends for improvement in airway hyperresponsiveness and FEV_1 in a dose- and time-dependent manner.[92] The anti-inflammatory effects of thiazolidinediones (rosiglitazone) in human airway smooth muscle cells might not be mediated by PPARs.[93]

IRMs AS ADJUVANTS FOR ALLERGEN IMMUNOTHERAPY

Toll-like receptors (TLRs) are cell-surface or intracellular receptors that recognize pathogen-associated molecular patterns (PAMPs) commonly conserved in bacteria, viruses, and some fungi. TLRs activate both innate and adaptive immune responses and can skew the cytokine balance from T_H2 toward T_H1, thereby inhibiting allergic diseases. To date, 11 TLRs have been identified in human subjects. CpG oligodeoxynucleotides are short, single-stranded synthetic DNA molecules that are unmethlyated and act as immunostimulants as PAMP motifs. The CpG PAMP is recognized by TLR9, which is constitutively expressed only in B cells and plasmacytoid dendritic cells. Class A CpG oligodeoxynucleotides stimulate the production of large amounts of type I interferons, the most important being IFN-α, and induce the maturation of plasmacytoid dendritic cells. Class A oligodeoxynucleotides are also strong activators of natural killer cells through indirect cytokine signaling. Class B oligodeoxynucleotides are strong stimulators of human B-cell and monocyte maturation. On TLR9 activation, plasmacytoid dendritic cells produce IFN-α, IL-6, and IL-10 and induce B-cell differentiation, IgG isotype switching, and antibody production.[94]

Tolamba (Dynavax Technologies, Berkeley, Calif) is a B-type CpG, ISS-1018, covalently linked to the major ragweed allergen Amb a 1. A phase 2 clinical trial in 25 adults with seasonal ragweed-induced allergic rhinitis showed improvements with 6 weekly subcutaneous injections before the ragweed season of the treatment year and a subsequent ragweed season.[95] However, a large, multicenter, phase 2 clinical trial did not meet its primary end point, leading to discontinuation of development.[96] An inhaled form of ISS-1018 significantly increased IFN-γ levels and IFN-γ–inducing gene expression but did not attenuate early- or late-phase asthmatic responses or reduce sputum eosinophil numbers or T_H2-related gene expression.[97]

A-type CpG with and without allergen has also been studied in both patients with asthma and those with allergic rhinitis. A study

of 20 patients with perennial rhinitis and house dust mite allergy demonstrated that subcutaneous allergen immunotherapy of house dust mite extract mixed with A-type ISS oligodeoxynucleotides contained in QbG10, a virus-like particle (Cytos Biotechnology, Zurich, Switzerland), over a 10-week period was well tolerated and reduced symptoms and skin test responses at least 38 weeks after treatment completion.[98] In a subsequent study CYT003-QbG10 was used without allergen in 80 patients with mild-to-moderate perennial allergic rhinoconjunctivitis. Subjects received 6 weekly subcutaneous injections of CYT003-QbG10 or placebo, and after 8 weeks, the treatment group had significantly improved total rhinoconjunctivitis symptom scores, and nasal allergen provocation results improved 100-fold.[99,100]

Sixty-three patients with persistent allergic asthma receiving inhaled corticosteroids received either 7 weekly subcutaneous injections of CYT003-QbG10 or placebo and were monitored over a 12-week period. During the run-in period, all subjects' symptoms were stabilized on beclomethasone, and 4 weeks later, beclomethasone was reduced by 50% and, if tolerated, reduced to zero after 4 more weeks. From weeks 6 to 12, the treatment group had significantly better combined symptom and medication scores than the placebo group and had stable FEV_1 compared with that seen in the placebo group at 12 weeks.[101]

TLR4 (CD284), which is expressed on the cell surface and with the adaptor molecule CD14, binds LPS. Monophosphoryl lipid A (MPLA) is derived from the active component of LPS and has been used as an adjuvant in the anthrax vaccine and investigated in patients with allergic respiratory diseases. Pollinex Quattro (Allergy Therapeutics, West Sussex, United Kingdom) contains pollen extract (grass, tree, *Parietaria* species, or ragweed) chemically modified by glutaraldehyde and adsorbed onto an L-tyrosine depot with an MPLA adjuvant. It has been used as a preseasonal ultra–short-course immunotherapy consisting of 4 weekly subcutaneous injections. Several studies have shown clinical and immunologic benefits, which appear to be better after the second year of treatment and are sustained after a 3-year course.[102-104] A recent study has demonstrated the potential of using MPLA to augment the clinical and biologic responses to sublingual immunotherapy in patients with grass allergy.[105]

Using a murine model of asthma, Duechs et al[106] studied the effects of several different TLR agonists on airway inflammation. Both TLR7 and TLR9 agonists reduced airway eosinophilia and reduced IL-4 and IL-5 levels. In contrast, TLR2 and TLR4 agonists potentiated both eosinophilia and neutrophilia. Only the TLR7 agonist did not enhance levels of proinflammatory cytokines in lung fluids. In a similar murine model of asthma, the synthetic TLR7 ligand agonist resiquimod (R848) attenuated allergic asthma through mechanisms that required regulatory T cells and was mediated by TGF-β.[107,108] Intranasal weekly administration of a TLR8 agonist has recently been shown to improve allergic rhinitis symptoms in an allergen environmental chamber study.[109]

ADVERSE EVENTS OF IRMs

As with any therapeutic treatment option, there are adverse reactions associated with IRMs.[110] A variety of adverse reactions have been seen with IRMs, including infections, hypersensitivity reactions, malignancies, and the cytokine release syndrome. Therapies that alter the host immune response to pathogens or

the inflammatory response might predispose the patient to infection. Immune therapies that interfere with proinflammatory cytokines, especially the TNF inhibitors (eg, etanercept and infliximab), have a 2-fold increased risk for serious respiratory tract infections that correlates with the dosage of the IRM. Comorbid conditions, concomitant immunosuppressive medications (eg, methotrexate), and the disease entity being treated (eg, rheumatoid arthritis) might enhance the risk of infection, even without the use of an IRMs. More serious than bacterial respiratory tract infections is the increased risk of opportunistic infections in patients treated with TNF inhibitors (eg, reactivation of latent tuberculosis, *Legionella* species, coccidioidmycosis, histoplasmosis, and *Listeria* species).[111] Most cases of tuberculosis were associated with reactivation of latent disease. Etanercept appears to be less of an issue with tuberculosis reactivation than infliximab. Thus in any patient treated with an IRM that affects the host response to infection, pharmacovigilance is needed.

IRMs can also be associated with an increased risk of malignancies. For example, TNF inhibitors have been associated with an increase in lymphoma and leukemia.[112] However, lymphoma is increased in patients with rheumatic diseases, even without IRMs.[113] Other adverse reactions by IRMs affect the neurologic system. Multiple sclerosis and other demyelinating conditions have been seen in patients treated with TNF inhibitors.[114] A form of progressive multifocal encephalopathy, a brain infection caused by reactivation of latent JC virus infection, has been seen with mAbs to VLA-4 (natalizumab) and efalizumab, an mAb that binds to the CD11a subunit of lymphocyte function-associated antigen 1. Other adverse events include systemic vasculitis with anti-TNF inhibitors and cardiac arrhythmias. The development of TNF inhibitors for asthma has been halted because of limited clinical efficacy in only a subset of patients and an unacceptable side effect profile leading to an unfavorable risk/benefit ratio.

Hypersensitivity reactions are a serious adverse reaction of many of the IRMs. There are several factors that affect the development of a hypersensitivity reaction to biologic agents, especially mAbs: source of the protein (mouse vs human), route of administration, treatment programs (ie, intermittent vs continuous), and concomitant use of immunosuppressive therapies (eg, methotrexate). Hypersensitivity reactions were observed with the first FDA-approved mAb, OKT3, an mAb that was made in the mouse.[115] Hypersensitivity reactions to infliximab, a chimeric mAb, commonly occur during the third or fourth infusion and can be variable, ranging from urticaria to anaphylaxis.[116] In clinical trials 17% to 21% of patients and as many as 60% of patients in other studies have antibodies against infliximab. True immediate hypersensitivity reactions are in the range of 2% to 3%.[117] Intermittent use of infliximab, such as in the treatment of Crohn disease, leads to greater development of antibodies. Acute infusion reactions might not be IgE mediated because reactions can occur on the first dose and most patients can tolerate subsequent infusions with premedication.[118] Interestingly, the use of concomitant immunosuppressive therapies decreases the likelihood of having a reaction. Systemic reactions are less common with etanercept and adalimumab, probably because of the route of administration (ie, subcutaneous) and the nature of the IRM molecule. Patients who have hypersensitivity reactions to infliximab can be safely switched to other anti-TNF inhibitors.[119] Immediate-type hypersensitivity reactions have been reported for a number of mAbs, including rituximab (5% to 10%),

trastuzumab (0.6% to 5%), omalizumab, natalizumab, basiliximab, abciximab, and cetuximab. All of these mAbs are either chimeric (majority) or humanized containing mouse protein. Another approach is desensitization. Brennan et al[120] share their experience with hypersensitivity reactions to mAbs and desensitization protocols.

Omalizumab is a humanized mAb that contains approximately 5% murine protein. Although immune complexes are formed with its target IgE, there is no complement activation. The mechanisms of anaphylaxis or anaphylactoid reactions to omalizumab are currently not known. One group suggested that the excipient Polysorbate 20 might be the cause of the reactions,[121] but further evaluation is needed to prove this. In clinical trials the prevalence of anaphylaxis to omalizumab was low (0.2%), although urticaria was seen more frequently (4.9% in a pediatric study).[122] An Omalizumab Joint Task Force reviewing the clinical trial data and the postmarketing surveillance data on reactions to omalizumab identified 35 patients and 41 episodes of anaphylaxis associated with omalizumab by using the criteria of the National Institute of Allergy and Infectious Diseases and the Food Allergy and Anaphylaxis Network[123] for a rate of 0.09%. Sixty-one percent of the reactions occurred in the first 2 hours after one of the first 3 doses. Five (14%) of the reactions occurred after the fourth or later doses and within 30 minutes. These data led the group to recommend a 2-hour direct observation period with the first 3 treatments and 30 minutes thereafter for subsequent treatments. It was also recommended by this task force that all patients should be educated on the signs and symptoms of anaphylaxis and in the use and availability of the epinephrine autoinjector.[17] Cruz et al[124] studied the safety of omalizumab in patients with asthma at high risk for intestinal parasites. Omalizumab therapy was safe and well tolerated but was associated with a slight increase in the incidence of helminth infections.

Often confusing with these reactions to IRMs is their occurrence with the first infusion. Although some of these reactions are not immune mediated, others appear to be consistent with a hypersensitivity reaction and even an IgE-mediated process. The latter could be due to a prior sensitivity and cross-reactive antigenic determinants. Such is the case with hypersensitivity reactions to cetuximab, a chimeric mouse-human IgG$_1$ mAb against the epidermal growth factor receptor, in which pre-existing IgE antibodies to the oligosaccharide galactose-α-1,3-galactose react against the glycosylation site on the Fab portion of the heavy chain of the cetuximab molecule.[125] Interestingly, the origin of these cross-reacting IgE antibodies to galactose-α-1,3-galactose might be related to prior tick bites.[126]

The cytokine release syndrome is a constellation of signs and symptoms ranging from flu-like symptoms to shock and is mediated by cytokine release, complement activation, and neutrophil activation, also known as the cytokine storm. Cytokine storms can occur in patients with a number of infectious and noninfectious diseases. This adverse response to IRMs was first observed with the administration of OKT3 (muromonab-CD3) in renal transplant recipients. The pathogenesis of this adverse event from mAbs is related to the activation of T cells with the release of proinflammatory cytokines to produce a systemic inflammatory response. It has also been described in patients receiving rituximab with the first infusion, especially in patients treated for malignancies. The use of an anti-CD28 mAb (TGN1412) in a phase 1 trial underscores the issue of translating murine
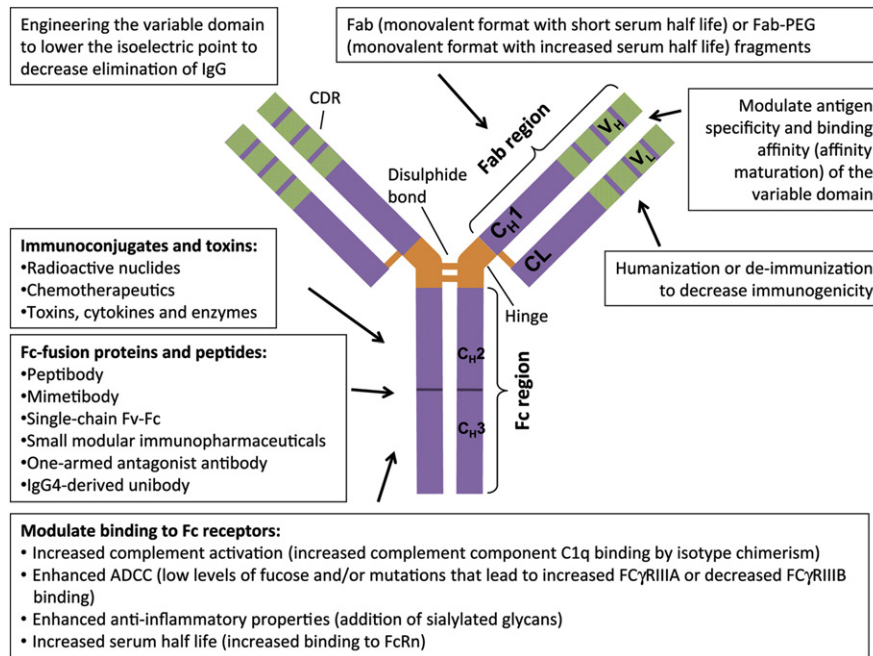
FIG 2. Strategies to improve the function of mAbs based on structure-function relationships. Adapted from Beck et al.[128]

experimental data to human clinical trials. This mAb is a superagonist that in the murine model expands $CD4^+CD25^+$ regulatory T cells and had a potential use in the treatment of autoimmune disorders. However, when 6 healthy young male volunteers received this mAb, within 90 minutes, all had a systemic inflammatory process characterized by the release of proinflammatory cytokines and a constellation of systems consistent with a cytokine storm.[127] This experience underscores the importance of being very careful in extrapolating preclinical data from an experimental model to human subjects.

THE FUTURE OF IRMs

We have come a long way since Kohler and Milstein developed their system for the production of mAbs. Monoclonal antibodies were initially all murine protein, then chimeric antibodies, then humanized antibodies in which only 5% was murine protein, and finally fully human mAbs thanks to advances in molecular biology and genetic engineering (Fig 1). A number of strategies have been developed to improve IRMs, especially mAbs. For mAbs with clinically validated targets, newer mAbs (second or third generation) are directed at the same antigen (ie, cell-surface molecule) but might target a different epitope or have improved variable domains to decrease immunogenicity, a higher affinity, or changes in the Fc domain to improve Fc function or provide a longer half-life (Fig 2).[128] As we understand more about the pathogenesis and immunobiology of diseases such as asthma, new targets will be identified for treatment. Advances in structure-function relationships have led to changes in antigen binding, stability, and pharmacokinetics. Molecular and genetic engineering has led to advances in affinity maturation and enhanced target specificity. The properties of the Fc domain can be modulated by altering the glycosylation sites to regulate the anti-inflammatory properties by using site-directed mutagenesis

to enhance antibody-dependent cellular cytotoxicity. Bioengineering the Fc domain can increase complement activation or increase the serum half-life by increasing the binding properties to the FcRn receptor. Another innovative approach is the engineering of bispecific antibodies that target more than 1 cell (Fig 3). For example, catumaxomab binds to both epithelial cell adhesion molecules on tumor cells and CD3 on effector T cells.[129] A related approach is the dual variable domain IgG (DVD-IgG) technology in which the variable domains of 2 already characterized mAbs are joined to form a single functional, dual-specific, tetravalent IgG-like molecule.[130] Advancements are also being made to decrease production and processing costs to make these IRMs more cost effective. Changes in the regulatory environment (eg, FDA policies) might lead to the faster approval and cheaper biosimilar IRMs that have come off patent. The next 10 years should be exciting and productive as advances in molecular biology and bioengineering are applied to biologic IRMs to improve their clinical efficacy by optimizing their design and developing more cost-effective production systems and purification processes. Ultimately, the production of lower-molecular-weight IRMs with good risk benefits and less cost will be most useful.

Although there are many advances in the bioengineering of mAbs, other approaches are also advancing the targeting of specific molecules or RNAs.

SUMMARY

Five percent to 10% of patients with asthma have severe disease that is not responsive to mainstream controller medications. Only approximately 13% of this group meet the criteria for treatment with omalizumab, the only available FDA-approved IRM approved for asthma. Clearly, as discussed above, asthma is a complex variable heterogeneous disease. Lötvall et al[8] describe
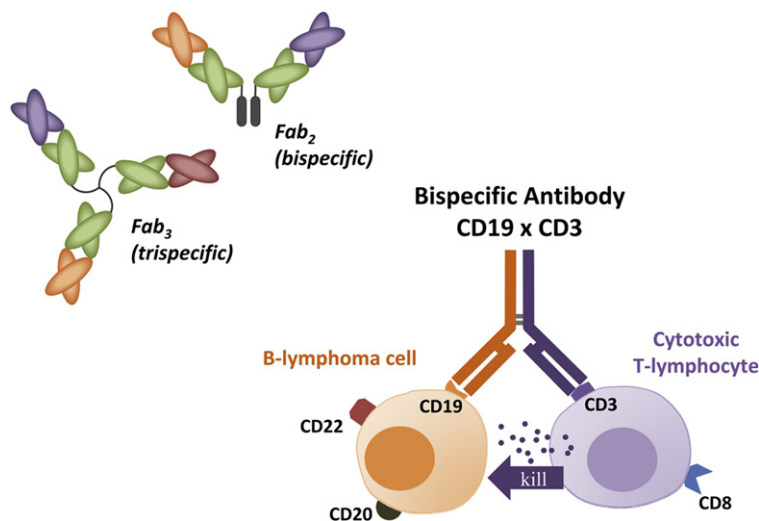
FIG 3. Examples of a bispecific antibody that binds to 2 different epitopes. These epitopes can be to the same antigen or pathogen or to 2 different targets (eg, on 2 different cells to trigger a specific function).

their approach in dividing asthma into distinct entities based not only on clinical presentations but also specific mechanisms, such as asthma endotypes.

Although the use of IRMs in the treatment of asthma and atopic disorders has not been as successful as anticipated by many or compared with their use in patients with rheumatic diseases, there have been some advances in our understanding of how to use these IRMs in disease endotypes. Although many of the studies with a variety of IRMs have shown little clinical efficacy, subset analysis of patient groups who exhibit certain characteristics, such as sputum eosinophilia, show improved primary end points and clinical efficacy that have led to new studies with a more defined patient population. A good example is the study of anti–IL-5 (mepolizumab). Recent studies of patients with eosinophilic refractory asthma[44] and in patients with prednisone-dependent asthma with sputum eosinophils[41] have been encouraging on the use of anti–IL-5 in certain asthma endotypes. At the same time, we must continue to be vigilant for adverse events and safety while studying these IRMs, as experienced by Wenzel et al[131] in a recent study of golimumab, an anti-TNF mAb that had an unfavorable safety profile.

Advances in the bioengineering of mAbs, fusion proteins, and small molecules that can be taken orally will be important steps in improving outcomes. For example, mAbs, perhaps bispecific antibodies, fusion proteins, or combination biologics that target more than 1 cytokine receptor or cytokine, such as pitrakinra,[26] will be more successful. The biggest issue is identifying asthma endotypes to tailor the correct IRM and to evaluate carefully the best primary outcome for defined patient subpopulations. The development of useful biomarkers is critical to identify patient-specific therapies and achieve these therapeutic goals. A case in point is the study of an anti–IL-13 mAb (lebrikizumab) using blood eosinophil counts and serum periostin levels as biomarkers to identify potential asthma endotypes that would respond to this treatment approach.[29]

As these clinical trials point out, elucidating the pathobiology and immunology of these patient subpopulations is a critical strategy for success. The development of new biomarkers will be critical in identifying these patient endotypes for more appropriate use of specific IRMs. Novel therapies must be directed at specific asthma endotypes if these new treatment modalities are going to be clinically efficacious and brought from the bench to the bedside.

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