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Immunoglobulin E and Allergy: Antibodies in Immune Inflammation and Treatment

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ABSTRACT The pathogenic role of immunoglobulin E (IgE) antibodies in triggering and maintaining allergic inflammation in response to allergens is due to the binding of multivalent allergens to allergen-specific IgEs on sensitized effector cells. These interactions trigger effector cell activation, resulting in release of potent inflammatory mediators, recruitment of inflammatory cells, antigen presentation, and production of allergen-specific antibody responses. Since its discovery in the 1960s, the central role of IgE in allergic disease has been intensively studied, placing IgE and its functions at the heart of therapeutic efforts for the treatment of allergies. Here, we provide an overview of the nature, roles, and significance of IgE antibodies in allergic diseases, infections, and inflammation and the utility of antibodies as therapies. We place special emphasis on allergen-IgE-Fcε receptor complexes in the context of allergic and inflammatory diseases and describe strategies, including monoclonal antibodies, aimed at interrupting these complexes. Of clinical significance, one antibody, omalizumab, is presently in clinical use and works by preventing formation of IgE-Fcε receptor interactions. Active immunotherapy approaches with allergens and allergen derivatives have also demonstrated clinical benefits for patients with allergic diseases. These treatments are strongly associated with serum increases of IgE-neutralizing antibodies and feature a notable redirection of humoral responses towards production of antibodies of the IgG4 subclass in patients receiving immunotherapies. Lastly, we provide a new perspective on the rise of recombinant antibodies of the IgE class recognizing tumor-associated antigens, and we discuss the potential utility of tumor antigen-specific IgE antibodies to direct potent IgE-driven immune responses against tumors.

IMMUNOLOGICAL MECHANISMS OF ALLERGIC DISEASES AND THE ROLE OF IgE ANTIBODIES

Clinical Perspective on Allergic Inflammation

Allergic inflammation, caused by development of an allergen-induced immune response, is largely driven via immunoglobulin E (IgE)-dependent mechanisms. It manifests clinically as asthma, rhinoconjunctivitis (more commonly known as hay fever), allergic skin inflammation (the main example of which is atopic dermatitis), food allergy, urticaria, and/or anaphylaxis, with several known disease variants caused by different underlying cellular and molecular mechanisms (1). Increased levels of circulating IgE, allergen-specific IgE reactivity profiles

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measured with radioallergosorbent tests and positive skin prick tests for specific allergens, together with auxiliary ex vivo and in vitro mast cell and basophil activation functional readouts, support the importance of IgE antibodies in the clinical manifestation of allergies (2, 3). Allergic inflammation can be local (that is, within the target organ), as is the case for allergic rhinoconjunctivitis and allergic asthma, or systemic, as is the case for anaphylaxis. The etiology of allergic immune responses has been shown to be influenced by several factors, including genetic susceptibility (4), route of exposure, dose of the allergen, and in some cases, structural characteristics of the allergen (5).

The allergic inflammatory cascade is thought to have evolved from the natural immune defenses to parasite and worm infections. This response can provide swift protection against these organisms but also participates in wound healing following tissue damage accrued as a result of the infection or accumulation of toxins. The same inflammatory cascades, originally evolved to neutralize invading parasites, are activated in response to innocuous antigens such as house dust mite and pollen proteins in the context of allergic diseases, triggering mast cell degranulation and eosinophil inflammation to the sites of allergen challenge. Furthermore, acute protective immune responses to infection evolve into chronic inflammatory cascades with long and persistent exposure to allergens. The consequences are persistent inflammation, tissue remodeling, and undesirable pathologies (6).

IgE Antibodies Activate Immune Responses in the Presence of Allergens

The discovery and characterization of IgE, culminating in the independent descriptions of this class of antibody by Ishizaka et al. and Johansson and Bennich, arguably represents the most crucial advance in our understanding of the immunological basis of allergic disorders (7, 8). Allergic inflammation is characterized by IgE-dependent activation of mast cells and an infiltration of inflammatory cells to sites of allergen challenge, orchestrated by increased numbers of activated $CD4^+$ T helper type 2 (Th2) lymphocytes.

Production of allergen-specific IgE requires that allergens are taken up by dendritic cells (DCs), monocytes, B cells, or other antigen presenting cells (APCs), which, in the presence of cytokines such as interleukin-4 (IL-4) or IL-13 present the processed antigens to cognate naive T cells that then acquire a Th2 cell phenotype (9). Th2 cells then engage cognate B cells through both B cell major histocompatibility complex class II and costimulatory molecules and secrete IL-4 and IL-13, inducing B cells to undergo class-switch recombination, resulting in the variable, diverse, and joining segments that were initially linked to another constant (C) region in the immunoglobulin heavy chain locus to instead be linked to the Cε region (10). Class-switch recombination can also be induced by IL-4 and/or IL-13 derived from cells other than Th2 cells, which may include mast cells and basophils (11). Allergen sensitization was previously thought to occur primarily in lymphoid germinal centers; however, IgE-producing B cells that undergo clonal selection and affinity maturation can also be generated locally at sites of antigen challenge such as the respiratory mucosa, gastrointestinal tract, and lymph nodes of individuals with food allergy, seasonal or perennial allergic rhinitis, or atopic or nonatopic asthma (11).

A Glance at IgE Receptors

IgE antibodies have two known cell surface receptors, FcεRI (the high-affinity receptor) and CD23 (otherwise known as FcεRII, the low-affinity receptor) (10). Each Fcε receptor recognizes a distinct epitope located on opposite sites of the Ce3 domain of the IgE constant region, and binding of one receptor to this domain prevents binding of the other to IgE (12). IgE antibodies also recognize the IgE- and FcεRI-binding protein galectin-3 (10).

FcεRI, which has affinity for IgE in the nanomolar range (K_a = 10^8 to 10^{10} M^{-1}) is expressed on basophils and mast cells as an αβγ2 tetramer and on Langerhans cells, myeloid DCs, plasmacytoid DCs, monocytes, and eosinophils as an αγ2 trimer (13). The α subunit has two extracellular immunoglobulin domains, which form the binding site of IgE to the receptor, and regulates movement of the receptor from the endoplasmic reticulum, where the subunits of the receptor are assembled to the cell surface membrane via an immunoreceptor tyrosine-based activation motif. The γ chain which is shared with FcγRs is necessary for receptor assembly and cell surface expression, while the β subunit is thought to enhance receptor functions and to also be responsible for enhanced receptor (αβγ2 tetramer) expression on the surface of mast cells and basophils. The downstream signaling events that culminate in degranulation in mast cells and basophils are mediated by the associated β and γ subunits.

The low-affinity receptor, CD23, can be induced on a broad range of immune cells, such as activated B cells, activated monocytes and macrophages, eosinophils, natural killer T cells, T cells, follicular DCs, and platelets, and also on nonimmune cells, such as airway epithelial cells and smooth muscle cells (10, 14, 15). Unlike

the high-affinity receptor, the low-affinity receptor, CD23, displays a micromolar affinity (K_a = 10^7 to 10^8 M^{-1}) for IgE as a monomer, but trimeric fragments are released from the cell membrane by metalloproteases and bind with 10 nM avidity (16). The CD23a splice variant is exclusively expressed on B cells activated by antigen, and it plays a key role in antigen endocytosis, processing, and presentation and in the regulation of IgE synthesis. Expression of the CD23b form can be triggered by IL-4 on a variety of effector cells, including B cells and macrophages. Engagement of CD23 by IgE-antigen complexes is thought to induce nitric oxide synthase and the release of cytokines such as tumor necrosis factor alpha (TNF-α) by macrophages, and these events have been reported to participate in phagocytosis and killing of parasites (17, 18).

Galectin-3, the beta-galactoside-binding lectin which recognizes both IgE Fc and FcεRI, exists in a secreted form and is also stored intracellularly. Its expression is associated with activated IgE immune cells such as monocytes/macrophages, DCs, mast cells, and eosinophils and also with B cells following stimulation by IL-4 and CD40-CD40L engagement (19).

IgE Effector Cells and Their Roles in IgE Responses

Mast cells: hallmarks of IgE-driven allergic response

The hallmark of an acute allergic response, mediated by allergen-IgE-FcεRI complexes cross-linking FcεRI on the surface of mast cells, is immediate (type I) hypersensitivity. Cross-linking can occur when the targeted antigen is multivalent, as two or more receptors need to be engaged to activate the ensuing signaling cascade, although the presence of cytokinergic IgEs capable of activating mast cells in the absence of multivalent antigen have been reported (20). Cross-linking of IgE-FcεRI complexes on the mast cell surface by allergens leads, within minutes, to the so-called "early phase" of the allergic response, involving mast cell degranulation and the rapid release of preformed vasoactive amines (mainly histamine), lipid mediators (such as prostaglandin D, platelet-activating factor, leukotriene C4 [LTC4], LTD4, and LTE4), chemokines (CXC-chemokine ligand 8, CXC-chemokine ligand 10, CC-chemokine ligand 2 [CCL2], CCL4, and CCL5), and cytokines (such as IL-4, IL-5, and IL-13). Mast cell activation plays a key role in generating the symptoms of allergy; the cytokines and chemokines liberated in this early phase initiate the "late phase," which peaks some hours later and involves the recruitment and activation of inflammatory cells (neutrophils, followed by eosinophils, monocytes, and lymphocytes) at sites sensitive to allergen. Similarly, but without overt symptoms, allergens activate the IgE-sensitized APCs, which in turn promote de novo immunoglobulin production by B cells, thereby maintaining mast cell and APC sensitization (10).

APCs trigger Th2 responses and IgE inflammation

Th2 cells are recruited and activated at the sites of allergic inflammation. A key research focus aims to understand the mechanisms by which allergens regulate Th2 cells through antigen presentation by professional APCs such as DCs. It has been proposed that allergic inflammation is initiated following allergen-epithelial cell contact, leading to the promotion of Th2 immunity via the production of a number of cytokines. Of key importance among these is epithelial cell-derived thymic stromal lymphopoietin, which conditions DCs to favor Th2 induction (21), while other epithelial cell-derived cytokines, namely IL-25 and IL-33, are also capable of inducing and maintaining Th2 immunity (22, 23). In addition, by secreting the chemokines CCL17 and CCL22, DCs possess definitive roles in the recruitment and activation of Th2 cells, while other cells secrete IL-4 which is necessary for Th2 cell differentiation (24). There is increasing evidence that basophils express FcεRI, major histocompatibility complex class II, and costimulatory molecules CD80/CD86, may participate in antigen recognition and processing, and can induce Th2 cell differentiation through the release of IL-4 (25, 26, 27). Other cells may also possess antigen presentation properties and can also secrete Th2 cytokines when activated by IL-25 and IL-33; these include mast cells, macrophages, eosinophils, and natural helper cells (28).

B cells and CD23 participate in antigen presentation

Allergen-IgE complexes bound to CD23 expressed by allergen-activated B cells can facilitate antigen presentation to T cells. The process of antigen presentation by way of CD23 is termed facilitated antigen presentation (FAP). The association between CD23 and HLA-DR in the cell membrane is involved in the trafficking of allergen-IgE-CD23 complexes to endosomes, where the allergen-derived peptides are loaded onto the HLA-DR molecules for presentation at the B-cell surface (29). An antigen-activated B cell expressing CD23 can simultaneously process unrelated antigens through FcRs and cause epitope spreading to other antigens. Indeed, CD23-mediated FAP is known to be as efficient as

FcR-mediated antigen presentation by DCs (30), orders of magnitude more efficient than B-cell internalization via surface immunoglobulin (the BCR).

B cells in draining lymph nodes participate in allergic inflammation through IgE synthesis. However, they are also present in the respiratory mucosa, and their capacity to produce IgE locally in tissues has been demonstrated (31). Following exposure to allergens, production of IgE is triggered by key Th2 cytokines IL-4 and IL-13, with IL-9 participating by enhancing IgE synthesis in situ (32, 33). Regulatory functions have also been attributed to B cells through secretion of IL-10, but the role of different subsets of B cells in allergic diseases remains to be determined (6).

Monocytes and eosinophils mediate IgE effector functions and tissue remodeling

Monocytes and eosinophils are among the IgE effector cell types that are drawn to sites of allergic inflammation. IgE upregulates the expression of FcεRI, whereas IL-4 and IL-13 can stimulate the expression of CD23 by these cells. This in turn arms the cells for functions such as clearance of antigen-IgE complexes, and killing and phagocytosis of pathogens (for example, helminth parasites) and tumor cells that bear "foreign" antigens (34, 35). In carrying out their functions, monocytes and eosinophils inevitably also inflict some damage on bystander cells in the tissue. This side effect may ultimately contribute to tissue remodeling and exacerbates the symptoms of chronic asthma. The IgE-binding protein galectin-3, which is expressed by monocytes, has both IgE-dependent and IgE-independent proinflammatory activities in the allergic response. Its expression is elevated in peribronchial monocytes, and it is released into the extracellular space. It contributes to cell survival and activation and to the retention of cells in the extracellular matrix in allergic inflammation (10). Galectin-3 also enhances the hypersensitivity of mast cells and the phagocytic activity of monocytes, perhaps by cross-linking additional IgE and FcεRI molecules on these cells when they have engaged their specific targets. The pentameric molecule galectin-3 may by itself contribute through FcεRI to airway smooth muscle cell contractile responses and thus to airway hyperresponsiveness and remodeling in asthma.

Interactions between Allergens, IgE, and FcεRI Trigger Hypersensitivity Responses

The high-affinity IgE receptor, FcεRI, is of primary interest to researchers investigating allergy, since its activation through engagement of IgE-multimeric antigen complexes mediates allergic reactions and could in some instances trigger anaphylactoid responses (36). Disrupting IgE-FcεRI interactions is expected to reduce IgE-mediated allergic responses and may result in loss of allergic inflammatory cascades, including anaphylactic functions. Mice lacking FcεRI showed normal development and differentiation of mast cells, B cells, and T cells, as well as a normal level of expression of the low-affinity IgE receptor, CD23 (37). However, these mice were resistant to systemic and cutaneous anaphylaxis induced by intravenous and subcutaneous injection of allergen-specific IgE and the allergen shortly afterwards. These data demonstrated that the interaction between IgE and the FcεRI could be interrupted to prevent escalation of the allergic response.

Upon multivalent antigen-IgE complex binding to the receptor, cross-linked FcεRI is mobilized in lipid rafts, where downstream signaling events potentiated by the associated β and γ chains activate phosphorylation by Src kinases (38), leading to degranulation, release of proinflammatory mediators, and the onset of allergic responses. In mast cells, these events are mediated through activation of RhoGTPases and mitogen-activated protein kinase pathways (39, 40). As well as the activation of signaling cascades to promote degranulation, the receptor also has sequences which interact with the actin cytoskeleton and microtubules, initiating signaling cascades that are calcium dependent and calcium independent, respectively (41, 42).

The central role of FcεRI signaling in triggering mast cell activation renders this receptor an attractive target for therapeutic approaches, which include neutralizing monoclonal antibodies.

Allergen-Specific IgG Antibodies Can Trigger Hypersensitivity Responses in Murine Models

IgE antibodies can mediate allergies and anaphylactic reactions, and several cell types have been described to be involved in these processes; among them are mast cells, basophils, and macrophages, which express Fcε receptors, and also neutrophils, which do not express Fcε receptors. Interestingly, studies with mice have demonstrated that effector cells are not exclusively activated by the combination of IgE, its high-affinity receptor, and antigens and that IgG antibodies can trigger cell activation and degranulation which can lead to anaphylactoid responses. It has been reported that active systemic anaphylaxis (ASA) can be induced through activation of FcγRs in vivo via IgE-independent effector mechanisms in mice (43, 44). Human neutrophils transferred into mice that lack active IgG Fc receptors on

mouse immune cells were described to restore ASA. FcγRIIA and FcγRIIIB are expressed on human neutrophils, but only FcγRIIA can bind mouse IgG, indicating that this receptor was responsible for restored ASA after human neutrophil transfer into mice (45, 46). In support of the role of FcγRIIA in anaphylactic reactions, a recent study using an FcγRIIA transgenic mouse model demonstrated that induction of FcγRIIA engagement and signaling was sufficient to trigger active and passive anaphylaxis as well as airway inflammation. Additionally, blocking FcγRIIA in the absence of IgE abolished the reactions induced by IgG antibodies in vivo (47). Further investigations revealed that IgG1 induced passive systemic anaphylaxis by activating basophils, whereas IgG2 induced passive systemic anaphylaxis via neutrophils, while ASA depended on monocytes/macrophages or neutrophils and not on mast cells and basophils (47, 48).

Thus, emerging evidence in murine models suggests that IgG and FcγR complexes contribute to allergic and anaphylactoid processes, although the precise requirements and pathways involved need to be elucidated.

IgE ANTIBODIES IN INFECTIOUS DISEASES

IgE Mechanisms of Protection in Parasitic Infections

Besides their critical role in allergy, IgE antibodies play a key physiological role in immunity against multicellular parasitic infections by a number of different mechanisms and via a number of IgE receptor-expressing cell types (49). The first in vivo evidence that IgE antibodies could be an essential component of the protective immunity against parasites was provided by passive transfer of monoclonal IgE antibodies directed against schistosomes (50). In this study, a rat monoclonal IgE antibody raised against *Schistosoma mansoni* afforded a significant level of protection against a challenge infection with *S. mansoni* when passively transferred into naive recipient rats. Furthermore, in another study, induction of resistance to infection by adoptive transfer of eosinophils or platelets bearing IgE indicated that the presence of IgE on these effector cells was crucial (51).

Subsequently, support for a role of IgE in parasite immunity was found when it was demonstrated that human eosinophils, platelets, and macrophages could harness IgE in vitro to mediate cytotoxicity and phagocytosis of *Schistosoma mansoni* or *Leishmania major* via FcεRI and CD23, respectively (17, 18, 52). These observations were subsequently established to be relevant to human immunity when epidemiological studies with *Schistosoma haematobium* provided evidence that host protection against reinfection in *S. haematobium*-infected populations was associated with high levels of parasite-specific IgE (53), and subsequently, IgE antibodies against *Schistosoma mansoni* were shown to positively predict resistance against reinfection with this blood fluke (54).

More recently, studies have demonstrated evidence that IgE antibodies are capable of activating a different cell type, namely mast cells to induce elimination of parasites via the release of toxic granules (55). *Trichinella spiralis* infection induces intestinal mastocytosis and heightened IgE responses, and elimination of this parasite requires expulsion of the adult worms from the gut and destruction of the larval cysts deposited in the muscles (56). In IgE-sufficient animals, intense deposition of IgE around the necrotic larval cysts was demonstrated with associated accelerated removal of worms from the intestine and a reduction in the viability of larval parasites in muscle (56). Indeed, *T. spiralis* infection drove a marked splenic mastocytosis and elevated serum levels of mouse mast cell protease-1 (MMCP-1), consistent with a systemic expansion of mast cells driven by the parasite. This mast cell increase was dramatically attenuated in $IgE^{-/-}$ mice, implicating IgE antibodies in this mast cell protection from parasitic infections. Furthermore, protective roles for mast cells during *T. spiralis* infection have also been observed using mast cell-deficient mice and by antibody inhibition of the mast cell marker, c-Kit (56).

Hyper-IgE Syndrome and Sensitivity to Infections

Elevated serum IgE is a major hallmark of hyper-IgE syndrome (HIES), a rare primary immunodeficiency disease. Common clinical manifestations include eczema and skin abscesses, often described as "cold boils" (57). HIES is clinically subdivided into autosomal dominant HIES (AD-HIES) and autosomal recessive HIES (AR-HIES). AD-HIES is associated with additional somatic abnormalities of the vascular, connective tissue, and skeletal systems, whereas AR-HIES is limited to skin conditions, including eczema, and is frequently more severe (58). HIES is further classified according to underlying mutations in key proteins involved in intracellular signaling networks, namely STAT3 and DOCK8. Dominant negative mutations in STAT3 are more frequent in AD-HIES (59) and DOCK8 mutations are more frequent in AR-HIES (60, 61), which may be directly related to the clinical and underlying immunological differences observed. In terms of allergy, patients

with DOCK8 mutations frequently suffer from symptoms of atopic disease such as asthma (62). It has been suggested that despite elevated serum IgE being a feature of both forms of HIES, differences in mechanisms of IgE regulation may be apparent. Xiong et al. suggested that affinity maturation pathways of IgE-producing cells may differ between atopic and nonatopic HIES patients, and such investigation would be very informative (63).

Recurrent *Staphylococcus aureus* infections in the lungs and skin is common in HIES and, to a lesser degree, *Haemophilus influenzae* and *Streptococcus pneumoniae* infections (58). It has been suggested that increased susceptibility to infection in AD-HIES is a direct consequence of STAT3 mutations (59, 64). STAT3 regulates multiple families of cytokines, including IL-6, IL-21, IL-22, and IL-23, which are involved in the generation of Th17 cells (65, 66). Th17 cells are essential for clearance of bacterial and fungal infections via induction and expansion of neutrophilic and antimicrobial responses (67). Impaired Th17 immune responses are a likely explanation for recurrent infection in AD-HIES (68).

The precise role, if any, of IgE in recurrent infections in HIES is unknown. It is likely that elevated serum IgE in nonatopic HIES patients is simply a marker of deregulated immune responses, with a dominating Th2 lineage driving the generation of IgE cells. In addition, impaired STAT3 induction of IL-21, a critical negative regulator of IgE class switching and B-cell death, is evident in animal models of AD-HIES (69) and may largely contribute to the associated high serum IgE levels observed in patients with HIES. As allergy and asthma are common in patients with DOCK8 mutations, the role of allergen-specific IgE may be more prominent in both the underlying pathogenesis of AR-HIES and its role in recurrent infections. Recent reports demonstrate that in nasal polyps from patients with chronic rhinosinusitis, which are frequently associated with *Staphylococcus aureus* infections, ongoing local receptor revision and class switching to IgE occurs (70). It is possible that such events occur during *Staphylococcus aureus* infection in AR-HIES, which could suggest a mechanism of interplay between infection and allergy in AR-HIES.

Current strategies to prevent recurrent *Staphylococcus aureus* infection in HIES involve administration of antibiotics and regular steroid treatment (58). Treatment of a patient with HIES with omalizumab, an anti-IgE antibody that blocks binding of circulating IgE to its Fc receptors on effector cells, resulted in significant clinical improvement (71). This report indicates that targeting IgE in AR-HIES may prove to be a beneficial alternative to current treatment strategies.

Virus-Induced IgE Antibodies

Multiple reports demonstrate that viral infection can induce IgE production. Infection of human tonsil B cells with measles virus induced germ line epsilon expression (72), which suggests that direct viral infection triggers IgE class switch recombination. It has been suggested that virus-induced activation of B-cell antiviral protein kinase R drives virus-induced class switching to IgE, via cellular detection of viral double-stranded RNA (73).

In allergic disease, viral infection may increase production of allergen-specific IgE antibodies and so increase susceptibility to allergen-induced IgE reactions such as mast cell degranulation. In atopic asthma, respiratory viral infections induce over 80% of asthma exacerbations, and 60% of these are strains of rhinovirus (74, 75), also known as the "common cold virus." The risk of hospitalization is greatest in asthmatics infected with rhinovirus who are both sensitized and have been exposed to allergen (76, 77), indicating an additive effect of viral infection on underlying allergic inflammatory reactions. In addition, serum levels of total IgE (78) and dust mite IgE (79) are elevated during rhinovirus infection in atopic asthmatics, which suggests that rhinovirus infection increases susceptibility to IgE-mediated reactions. The precise mechanisms for how in vivo rhinovirus infection increases production of IgE are unknown; however, an advanced understanding of rhinovirus-induced inflammation has generated logical hypotheses. Both in vitro and in vivo rhinovirus infection enhance Th2-mediated airway inflammation in asthma (80, 81, 83). It is possible that rhinovirus-induced inflammation drives germinal center reactions within the bronchial mucosa. Local germinal center reactions could generate B cells within the lungs to class switch to IgE and induce differentiation into IgE-secreting plasma cells. It is possible that this excessive local production of IgE during rhinovirus infection could spill over into the circulation, giving rise to the detected increase in serum IgE during infection in asthma. In 1998, Rager et al. demonstrated that infection of Ramos B cell line with rhinovirus 14 and 16 strains (RV14 and RV16) induced germ line epsilon expression (73). Whether in vivo rhinovirus infection induces IgE class switching or B-cell proliferation and differentiation into IgE plasma cells would be informative.

The role of respiratory syncytial virus (RSV) in the development of asthma and allergies in children has been well established (84). Children with recurrent RSV infections developed high titers of serum IgE and tested

positive with allergen skin prick tests (85). The risk of developing asthma was significantly greater in these children, highlighting RSV as a major risk factor for the development of allergic asthma. RSV has been shown to induce production of RSV-specific IgE (86). Dakhama et al. demonstrated a positive correlation between RSV-IgE production and severity of airway hyperresponsiveness in mice (86).

Investigation into mechanisms of virus-induced IgE antibodies in allergic disease will prove very informative and could aid the development of novel preventative therapies for both virus-induced asthma exacerbations and the development of asthma.

ANTIBODIES IN THE TREATMENT OF ALLERGIES

Recombinant Antibodies Targeting IgE Interactions with Fcε Receptors

Designing therapeutic antibodies targeting IgE represents an approach for the treatment of patients with allergies. The primary aim of anti-IgE therapy is to reduce the amount of circulating IgE in patients and to neutralize the interactions between free IgE and immune cells responsible for mediating allergic reactions (87). One approach entails blocking free IgE from binding to its Fc receptors expressed on immune effector cells, such as basophils and mast cells, to prevent the release of inflammatory mediators, such as histamines and tryptases, from these cells which lead to hypersensitivity. Another would be to downregulate the production of IgE by B cells.

IgE-blocking antibodies for passive immunotherapy: omalizumab

There have been numerous anti-IgE antibodies in preclinical and clinical development, with omalizumab (Xolair) being the first licensed anti-IgE therapy approved for the treatment of allergic bronchial asthma in 2003 (88). Omalizumab represents an important breakthrough in the development of antibodies for anti-IgE immunotherapy in allergy. Omalizumab is a humanized IgG1 monoclonal antibody that recognizes an epitope in the Cε3 region of IgE, the IgE Fc region which contains the binding sites for both the high- and low-affinity IgE Fc receptors (10, 89). This anti-IgE antibody can block the binding of circulating IgE to both of its Fc receptors, which are expressed on the surfaces of various effector cells, thus preventing IgE-mediated allergic immune responses (Fig. 1).

Treatment with omalizumab also has been shown to result in downregulating the expression of FcεRI on immune cells (Table 1) (90, 91, 92). Notably, omalizumab is not able to bind to IgE in the complex with its receptors, possibly due to allosteric inhibition. It was recently shown that engagement of IgE with omalizumab triggers a lower degree of bending in IgE compared to that adopted through recognition of FcεRI, inducing a conformational change that is incompatible with FcεRI recognition (93). Due to these properties, the antibody is not capable of cross-linking IgE already bound to receptors on the surfaces of mast cells and basophils, and therefore, any unwanted immune cell activation and release of inflammatory mediators is avoided (94). Examination of cross-linking is essential in the design of IgE immunotherapy to ameliorate the risk of any acute allergic reactions such as type 1 hypersensitivity in patients (Table 1).

Anti-IgE therapy has widespread applicability in asthma. Currently, omalizumab is being evaluated for the treatment of many other IgE-mediated allergic diseases, including atopic dermatitis, seasonal allergic rhinitis, food allergy, and urticaria (Table 2). Although discontinued for clinical development, due to partnership agreements rather than clinical utility, the anti-IgE antibody talizumab (TNX-901) demonstrated the ability to increase the sensitivity threshold to peanuts, supporting the use of anti-IgE therapy for some food allergies to prevent mast cell/basophil degranulation (95). Talizumab can also inhibit the presentation of allergens, since it was shown to block allergen-CD23 complex formation on the surface of B cells, and can prevent proliferation of allergen-specific T cells (96).

Antibodies blocking CD23 functions and B cells

Another approach centers on targeting CD23 with the aim of regulating IgE production through blocking the functions of the low-affinity IgE receptor, CD23, on B cells. A prominent agent featuring this approach is primatized anti-CD23 macaque/human chimeric antibody, lumiliximab (IDEC-152), which was designed to modulate antigen presentation, reduce activation of Th2 responses, and hinder IgE production by B cells (97). This agent reached clinical evaluations for the treatment of allergic asthma, but it is now evaluated for use in hematological oncology indications such as chronic lymphocytic leukemia.

New anti-IgE antibodies are undergoing preclinical evaluations and development and include agents aimed at downregulating the production of IgE by B cells. One emerging strategy is the engineering of an antibody (8D6) which, like omalizumab, prevents the binding of free IgE to cell surface FcεRI but additionally interacts

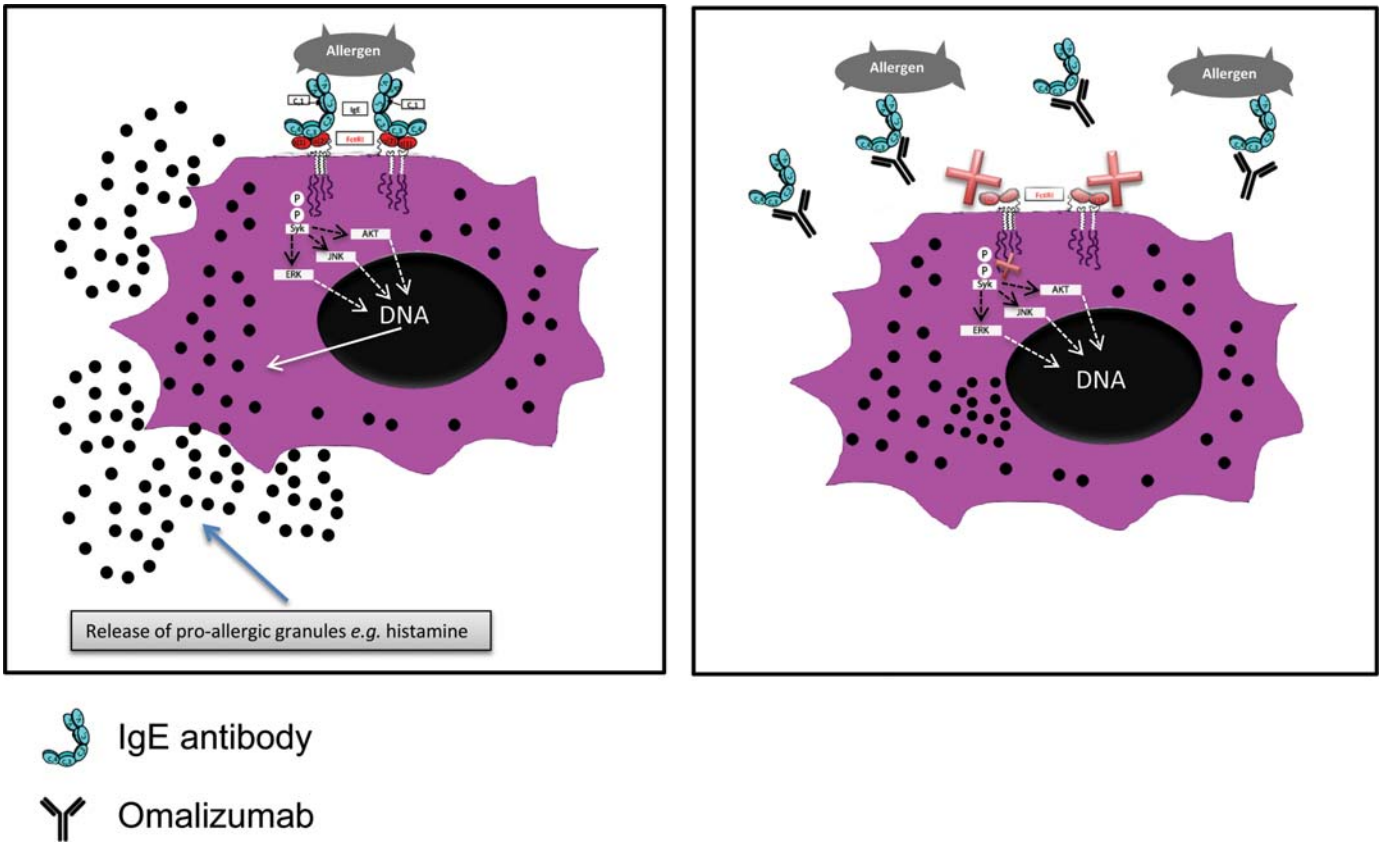

FIGURE 1 Omalizumab blocks IgE-mediated mast cell activation. The allergic response mediated by multivalent allergen-IgE-FcεRI complex formation on the surface of mast cells triggers cross-linking of FcεRI, leading to downstream signaling events potentiated by the β and γ chains (left). These entail phosphorylation (P) by Src kinases and cellular activation through RhoGTPases and mitogen-activated protein kinase pathways, leading to mast cell degranulation and the rapid release of a range of vasoactive amines (e.g., histamine), prostaglandins, leukotrienes, cytokines, and chemokines, inducing and maintaining allergic inflammatory responses. Omalizumab, a humanized IgG1 monoclonal antibody that recognizes an epitope in the Cε3 region of IgE, can block the binding of circulating IgE to FcεRI, sequestering free and allergen-bound IgE (right). These interactions prevent allergen-IgE-FcεRI complex formation on the surface of mast cells and interfere with the signaling cascades that trigger degranulation and the onset of IgE-mediated allergic inflammatory cascades. Syk, spleen tyrosine kinase; AKT, Ak strain thymoma serine/threonine-specific protein kinase; ERK, extracellular signal-regulated kinase; JNK, Jun N-terminal protein kinase; P, phosphorylation. doi:10.1128/microbiolspec.AID-0006-2012.f1

with CD23-bound IgE with the aim of also inhibiting IgE production on B cells, thereby controlling circulating IgE levels (98). Another approach encompasses an anti-IgE antibody, XmAb7195, with modified IgG Fc domains that feature increased affinity to the inhibitory FcγRIIB expressed on B cells and also the ability to suppress B-cell activation. Like omalizumab, this agent was recently reported to target the Cε3 domains of free IgE but to also cointeract with IgE in the complex with FcγRIIB on the surface of IgE-expressing B cells (99). This antibody was shown to suppress B-cell signaling and prevent maturation of B cells into IgE^+ plasma cells, reducing total IgE in circulation in vivo while neutralizing IgE in a manner similar to omalizumab. The two complementary properties resulted in prolonged efficacy for XmAb7195 compared to antibodies exerting only one of the two mechanisms.

Another strategy involved use of the anti-CD20 chimeric antibody rituximab, which has been approved for the B-cell malignancy non-Hodgkin lymphoma and has also demonstrated efficacy in some autoimmune disorders (100). The antibody was examined as a potential tool to eliminate B cells and therefore remove allergen-induced B-cell class switching and synthesis of IgE and also IgG autoantibodies, for applications in atopic dermatitis and urticaria, and anecdotal successes for individual patients treated with this agent have been reported (101, 102).

As demonstrated by the success of omalizumab and the other anti-IgE therapies undergoing preclinical and

TABLE 1 Interactions of omalizumab with IgE, the resulting mechanisms of action, and clinical effects of treatment in patients with allergies

Monoclonal antibody property	Mechanism	Biological function	Immunological effects	Clinical impact
Binds to the Cε3 region of free circulating IgE	Sequesters free IgE	Reduction of IgE titers in human sera and reduced levels of cell-bound IgE	Reduced mast cell and basophil activation, reduced antigen presentation	Reduced allergic symptoms/efficacy
	Prevents IgE-FcεRI interactions on cell surfaces	Decreased FcεRI density on circulating basophils	Decreased basophil/mast cell responsiveness upon challenge with IgE-allergen complexes	Reduced allergic symptoms/efficacy
		Decreased FcεRI density on APCs (e.g., DCs)	Inhibition of antigen processing and presentation to T cells, reduced Th2 cellular and cytokine responses	Reduced allergic inflammation/efficacy
	Prevents IgE-FcεRII interactions on cell surfaces	Inhibition of allergen-IgE-FcεRII complex formation Cross-linking and IgE-mediated endocytosis	Decreased antigen presentation to T cells Decreased de novo synthesis of IgE	Reduced chronic allergic responses/efficacy
Does not interfere with variable regions of IgE	Circulating IgEs of any specificity sequestered Blocks cell surface interactions of FcεRs with any IgEs	Blocks IgE-FcεR-IgE functions mediated by any allergen	Inhibition of allergic responses irrespective of allergen specificity	Broad applicability in allergic diseases
Binds to a Cε3 epitope in proximity to both binding sites recognized by FcεRI and FcεRII	Does not recognize IgE bound to cell surface IgE-FcεRs	Addition of antibody could not trigger FcεRI cross-linking and mast cell/basophil degranulation	Reduced potential for type 1 hypersensitivity responses that may lead to onset of anaphylaxis	Improved safety profile
High specificity for IgE Fc	Sequesters free IgE only	Reduction of IgE titers but no reduction of IgG	Normal immunity is unaffected	No off-target effects/safety

TABLE 2 Active phase II, III, and IV interventional clinical studies for antibody immunotherapies of allergic diseases in 2012[a]

Trial title	Intervention	Target	Phase	Indication	Primary endpoint(s)	Sponsor	Status
Oral immunotherapy combined with humanized monoclonal anti-IgE antibody Xolair (omalizumab) in the treatment of cow's milk allergy	MAb + OIT[b]: omalizumab + milk OIT	IgE	II	Milk allergy	Efficacy: percentage of subjects developing clinical tolerance to milk	National Institute of Allergy and Infectious Diseases (NIAID)	Recruiting
Peanut oral immunotherapy and anti-IgE for peanut allergy	MAb + OIT: omalizumab + peanut OIT	IgE	I/II	Peanut hypersensitivity	Efficacy: percentage of subjects developing clinical tolerance to peanuts	University of North Carolina (collaborator, Genentech)	Active, not recruiting
Effect of KB003 in subjects with asthma inadequately controlled by corticosteroids	MAb: KB003-04	GM-CSF[c]	II	Moderate-to-severe asthma	Safety, tolerability, and efficacy: effects on lung function measured by FEV1[d]	KaloBios Pharmaceuticals	Recruiting
A phase 2b, randomized, double-blind study to evaluate the efficacy of tralokinumab in adults with asthma	MAb: tralokinumab	IL-13	IIb	Asthma	Efficacy: asthma exacerbation rate	MedImmune LLC	Recruiting
Dose ranging pharmacokinetics and pharmacodynamics study with mepolizumab in asthma patients with elevated eosinophils	MAb: mepolizumab (SB-240563)	IL-5	II	Asthma	Pharmacokinetics, pharmacodynamics, immunogenicity analysis; effects on blood eosinophil levels, drug plasma levels	GlaxoSmithKline	Recruiting

Omalizumab in patients with moderate to severe persistent allergic asthma not adequately controlled despite GINA (2009) step 4 therapy	MAb: omalizumab	IgE	III	Persistent allergic asthma	Efficacy: change from baseline in mean morning PEF[e]	Novartis Pharmaceuticals (collaborator, Genentech)	Recruiting
A study of lebrikizumab in patients whose asthma is uncontrolled with inhaled corticosteroids and a second controller medication (LUTE)	MAb: lebrikizumab	IL-13	III	Asthma	Efficacy: rate of asthma exacerbations	Genentech	Recruiting
A study of lebrikizumab in patients with uncontrolled asthma who are on inhaled corticosteroids and a second controller medication (VERSE)	MAb: lebrikizumab	IL-13	III	Asthma	Efficacy: rate of asthma exacerbations	Genentech	Recruiting
Effect of Xolair on airway hyperresponsiveness	MAb: omalizumab	IgE	IV	Allergic asthma	Efficacy: reduction in abnormal increase in limitation to airflow in patients with asthma; decrease the amount of inflammation in the lungs	Creighton University (collaborator, Genentech)	Active, not recruiting

[a]Source: ClinicalTrials.gov; U.S. National Institutes of Health.
[b]MAb, monoclonal antibody; OIT, oral immunotherapy.
[c]GM-CSF, granulocyte-macrophage colony-stimulating factor.
[d]FEV1, forced expiratory volume in 1 s.
[e]PEF, peak expiratory flow.

clinical evaluations, monoclonal antibodies targeting IgE are emerging as important therapeutic modalities for the treatment of allergic diseases, while new approaches are being examined which aim to improve the efficacy of omalizumab. Other strategies with indirect effects on IgE production, such as those aimed at neutralizing B cells and targeting CD23, have been less successful to date.

Antibodies Targeting Key Cytokines, Chemokines, and Their Receptors

Th2-type cytokines (e.g., IL-4, IL-5, IL-9, and IL-13) are produced by mast cells, basophils, Th2 lymphocytes, and eosinophils but also by nonimmune cells such as lung airway epithelial cells. They play key roles in triggering and maintaining allergic inflammation and contribute to the resulting pathologies. Targeting cytokines associated with allergies with antibodies has been explored as a treatment option, and various strategies are being examined in preclinical studies and in clinical trials, with asthma being a major therapeutic indication for these efforts.

Production of IL-4 by basophils, mast cells, NK cells, or eosinophils and subsequently also by Th2 lymphocytes is linked to a number of processes in allergic inflammation, such as upregulation of FcεRI and CD23 on the surface of effector cells and class switching from IgM to IgE by B lymphocytes. Therefore, neutralizing IL-4 would be expected to inhibit IgE synthesis, formation of Th2-type T cells, and upregulation of IgE receptors. Preclinical studies in a murine model of asthma demonstrated that an anti-IL-4 antibody could inhibit IgE production, but this agent did not abrogate allergic inflammatory cascades in animal lungs (103). Phase I and II clinical studies in patients with asthma using a humanized antibody (pascolizumab, SB-240683) which blocks IL-4–IL-4 receptor interactions did not result in clinical improvements in patients (104).

By sharing a receptor with IL-4 in the form of IL-4R subunit α, IL-13 is thought to be involved in class switching to IgE but also has functions distinct from IL-4, such as regulation of epithelial cell maturation, muscle contractility, production of extracellular matrix proteins, and recruitment of monocytes, T cells, and eosinophils. Targeting IL-13 is presently an area of translational and clinical activity. Tralokinumab (CAT-354), a human IgG4 antibody which can neutralize IL-13, was associated with improved lung function when tested in patients with asthma, although results from phase IIb clinical studies are pending (Table 2) (105). Another agent presently undergoing clinical evaluations in phase III clinical trials is the IgG4 humanized anti-IL-13 antibody, lebrikizumab. Small improvements in lung functions were observed when this antibody was administered in asthmatics with inhaled glucocorticoid-resistant disease, and clinical responses were associated with increased levels of serum periostin, suggesting the merits of utilizing biomarkers to select patients most likely to respond (106).

Eosinophilia comprises an important component of the inflammatory infiltrate in asthmatic lungs, and increased levels of eosinophils are found in the blood of allergic subjects. Because of its role in potentiating activation and proliferation of eosinophils and their terminal differentiation in tissues, IL-5 constitutes an attractive therapeutic target for allergic conditions characterized by eosinophilia (107). Three humanized antibodies targeting IL-5 are in clinical trials for the treatment of eosinophilia-associated allergic conditions. Clinical studies of patients with hypereosinophilic conditions reported that treatment with mepolizumab (SB-240563) resulted in increased titers of serum IL-5 and IL-5 receptor, while reduced eosinophil activation and lower blood eosinophil levels were observed (108). One study demonstrated disease management with lower doses of corticosteroid treatment and reduced bronchial mucosal eosinophilia in asthmatic subjects receiving this antibody. Others reported reduced exacerbation of symptoms in asthmatics, with further clinical trials with this agent presently in progress (Table 2) (109, 110). Two other humanized antibodies, the anti-IL-5 antibody reslizumab (CTx55700) and the anti-IL-5 receptor-specific antibody benralizumab (MEDI-563), have demonstrated favorable safety profiles and reductions in eosinophil counts in patients. So far, clinical utility has not been proven for anti-IL-5 therapies, although clinical studies are still underway (111, 112, 113).

Studies of murine models of asthma demonstrated that IL-9 provides activatory and proliferative signals to mast cells, contributing to airway hyperresponsiveness and airway fibrosis. Targeting this cytokine with an antibody reduced these allergen-induced inflammatory symptoms, and antibody treatment was associated with reduced levels of known pro-fibrotic cytokines transforming growth factor β, vascular endothelial growth factor, and fibroblast growth factor 2 (114). A humanized antibody targeting IL-9 (MEDI-528) has been tested in clinical trials in healthy individuals and patients with asthma with some encouraging clinical responses (115).

Expressed by macrophages and mast cells following activation with different stimuli, including IgE-FcεRI associations, TNF-α triggers infiltration of immune effector cells in tissues and has a role in inflammatory

processes, including those associated with asthma (116). Anti-TNF-α antibodies tested in the clinic include the chimeric infliximab, the humanized certolizumab pegol, the human antibodies adalimumab and golimumab, and the soluble TNF-α receptor 2-Fc fusion protein, etanercept. All of these agents are expected to neutralize TNF-α. However, any moderate clinical responses observed have been overshadowed with reports of induction of opportunistic infections and cancer, which may reflect the importance of considering the complex balance between mounting natural protective immune functions and reducing allergic inflammatory environments when designing therapeutics for allergic diseases (117).

Active Allergen Immunotherapy Induces Neutralizing Antibody Responses in Patients

An attractive therapeutic direction entails strategies to interrupt IgE binding to allergens to reduce IgE-mediated responses in patients with allergies. For known allergens, it is possible to produce a vaccine that would reduce the inflammatory response of antigen-bound IgE binding to mast cells by disrupting the binding of the Fab region of IgE to allergenic epitopes, and this has formed the focus of recombinant allergen immunotherapy. This therapy has been examined now for decades; the availability of allergen sequences has led to the expression of recombinant allergens and production of synthetic allergenic peptides, and there now exists a database where these sequences have been deposited for comparison (118). Immunization with recombinant allergen induces the production of allergen-specific IgGs, which compete with IgEs for binding to the allergen, thus blocking downstream IgE cross-linking and suppressing allergic responses (119).

There are a number of subclasses of these recombinant and synthetic allergens. Of particular interest to those who study the allergic response stimulated by the cross-linking of IgE are the wild-type hypoallergens. These are designed to be allergen mimics and may consist of a fragment of the allergen, a mutant of the allergen, or the whole recombinant allergen. The net result of this subclass is reduced IgE activity in patients. The first clinical trial of the wild-type hypoallergen was with the Birch pollen allergen, Bet v 1, with patients who showed an allergic response to the crude extract. Application of rBet v 1, as well as fragments of the Bet v 1, showed efficacy in reducing histamine release in basophils, an important step in the progression of an allergic response (120). The same trial also showed that there was an increased production of allergen-specific IgG1 and IgG4 with both the full-length allergenic recombinant and smaller fragments, suggesting that a few allergenic residues are all that are required to induce a clinical regression of symptoms in patients. Not only was there a notable lack of histamine reaction to the use of recombinant therapies but there were also no T-cell-mediated toxic effects noted.

The efficacy of recombinantly expressed allergen has been characterized and shown to be capable of inducing desensitization to specific allergens. The Der p 2 allergen is the major allergen responsible for dust mite allergies, and there are a number of allergenic isoforms. One of these isoforms was selected and expressed in the yeast strain *Pichia pastoris* and as inclusion bodies in *Escherichia coli*, where they were refolded to a stable tertiary structure. The secondary structures of these recombinant products were compared to the native allergen, obtained from dust mites by circular dichroism, and showed that different structures were obtained for each of the products. However, in a mouse model, both of the recombinant products were capable of inducing desensitization to the Der p 2 allergen, despite differential structural properties. These findings suggest that potentially linear epitopes may be sufficient for allergenic vaccine therapy (121).

Based upon the success of recombinant allergens, rational design of allergenic epitopes was the next logical step, since, as far back as 1970, allergens were being chemically modified to reduce IgE binding capacity while retaining the ability to prompt the production of antigen-specific IgGs (122, 123). More recently, the use of recombinant technology yielded new tools with which to mutate specific residues that alter the activity of the allergen. An example of this approach was the use of mutated forms of the mouse allergen Mus m 1. The high resolution crystal structure of a domain of the allergen highlighted a potentially important residue (124). When this residue was mutated, in two single-point mutant forms of the allergen, it resulted in very different circular dichroism spectra, suggesting a change in conformation of the allergen based on altering this site. Both of the mutants had reduced ability to bind IgE and activate basophils but could be recognized by allergen-specific T cells. These findings suggest that site-directed mutagenesis retains the immunogenicity of these allergens while counteracting initiation of an allergic response.

In 2011, there were 11 clinical trials running in the United Kingdom using recombinant allergens to combat allergic diseases, with 4 of these being in phase III (125). The relative success of this therapy suggests that

targeting the interaction of the antibody with the allergen may be an effective therapeutic alternative to or complementary to the use of anti-IgE antibodies.

Triggering Protective Immunity with Immunotherapy—the Role of IgG4

Specific immunotherapy (SIT) involves the administration of allergens to achieve clinical tolerance, with the aim of easing the symptoms in patients with allergic conditions. Long-term clinical benefits of SIT persist even after discontinuation of therapy, indicating the involvement of a cellular memory component to therapy. The mechanism behind the therapy has extensively been studied but still remains a matter of research and debate.

Research on SIT has to date focused on altered T-lymphocyte responses and induction of allergen-specific IgG4 antibodies. Two individual patterns of change, which may occur sequentially, have been described. The first event is induced within 1 to 4 weeks and entails the generation of regulatory T cells secreting IL-10 and transforming growth factor β, accompanied by suppression of allergen-induced late cutaneous responses. Subsequently, around week 10 postchallenge, elevated serum titers of allergen-specific IgG4 and IgA are observed. Although these titers appeared proportional to the dose of administered antigen rather than to clinical improvements, recent studies reported that the functional capacity of IgG4 antibodies to block IgE functions correlates with clinical responses (126, 127). These events coincide with a decrease of allergen-specific IgE antibodies and a shift in the allergen-specific T-cell response from predominantly Th2 to Th1 (126).

Serum obtained from patients following SIT has been shown to inhibit allergen-IgE binding to B cells, an effect mediated largely by IgG4 antibodies in patient sera. This in vitro system introduced the idea that SIT triggers production of "blocking antibodies" that inhibit IgE functions, such as IgE-FAP on B cells. Similarly, basophil histamine release assays or basophil activation assays have demonstrated the functional ability of IgG4 to inhibit IgE-dependent activation and mediator release, either by competing with IgE for the antigen and/or by stimulating cell surface IgG-inhibitory receptors present on basophils and mast cells (127, 128). Interestingly, IgA antibodies could not block allergen-IgE binding to B cells. The contribution of IgA in the responses to SIT may lie in engagement of surface IgA receptors and release of the inhibitory cytokine IL-10, which may participate in the induction of immune tolerance to allergens (129).

The mechanisms triggering elevated IgE-neutralizing antibodies following SIT are not completely understood, although it is known that prolonged exposure to antigenic stimuli can direct production of IgG4 by B cells. B cells can switch from IgG4 to IgE but not vice versa, as these sections of the gene are spliced out during class switching. Thus, IgG4 must be produced by switching from IgM, IgG1–3, or IgA1 or by proliferation of preexisting $IgG4^+$ B cells during SIT. It has been suggested that Th2 cytokine environments with elevated levels of IL-10 can drive the differentiation of IgG4-switched B cells to IgG4-secreting plasma cells (130). In addition to IL-10, the immunoregulatory cytokine, IL-21, has been found to increase IgG4 production in vitro (131).

The IgE-neutralizing and -tolerogenic properties of IgG4 may be partly due to competing with allergen-specific IgE for antigen specificity. This would interrupt IgE-FcεRI-multivalent allergen complex formation on the surface of immune effector cells, preventing downstream signaling and effector cell activation (Fig. 2). However, since only a fraction of IgG4 antibodies are allergen specific after immunotherapy, other mechanisms may also be involved. van der Neut Kolfschoten and colleagues used recombinant IgG4 antibodies against grass and cat allergens, which when coinjected in a mouse graft, became bispecific in a process termed Fab-arm exchange. Although this process was not observed when antibodies were combined in vitro, these findings raise the possibility that IgG4 antibodies may also operate by Fab-arm exchange (132). If this is true in patients receiving allergen immunotherapy, IgE/IgG4 bispecific antibody formation may reduce the affinity of IgE antibodies for allergens and interrupt IgE-FcεRI-multivalent allergen complex formation on the surface of immune effector cells. These properties would therefore moderate IgE-mediated inflammatory cascades. This latter possible mechanism by which IgG4 antibodies may function would be consistent with the observations that IgE effector cells such as eosinophils, mast cells, or basophils are reduced after SIT against house dust mite and grass pollen (133). An additional mechanism by which SIT can tolerize allergic immune responses lies with the ineffective Fc-mediated functions of IgG4 in lacking complement activator properties and mediating ineffective FcR signals, reducing the capacity of this antibody class to trigger cytotoxicity or phagocytosis.

Additionally, involvement of IgG4 and also IgA antibodies in natural induction of tolerance is supported by reports of increased allergen-specific IgG4 serum levels in patients who spontaneously become tolerant to cow's milk and from findings that these serum levels are

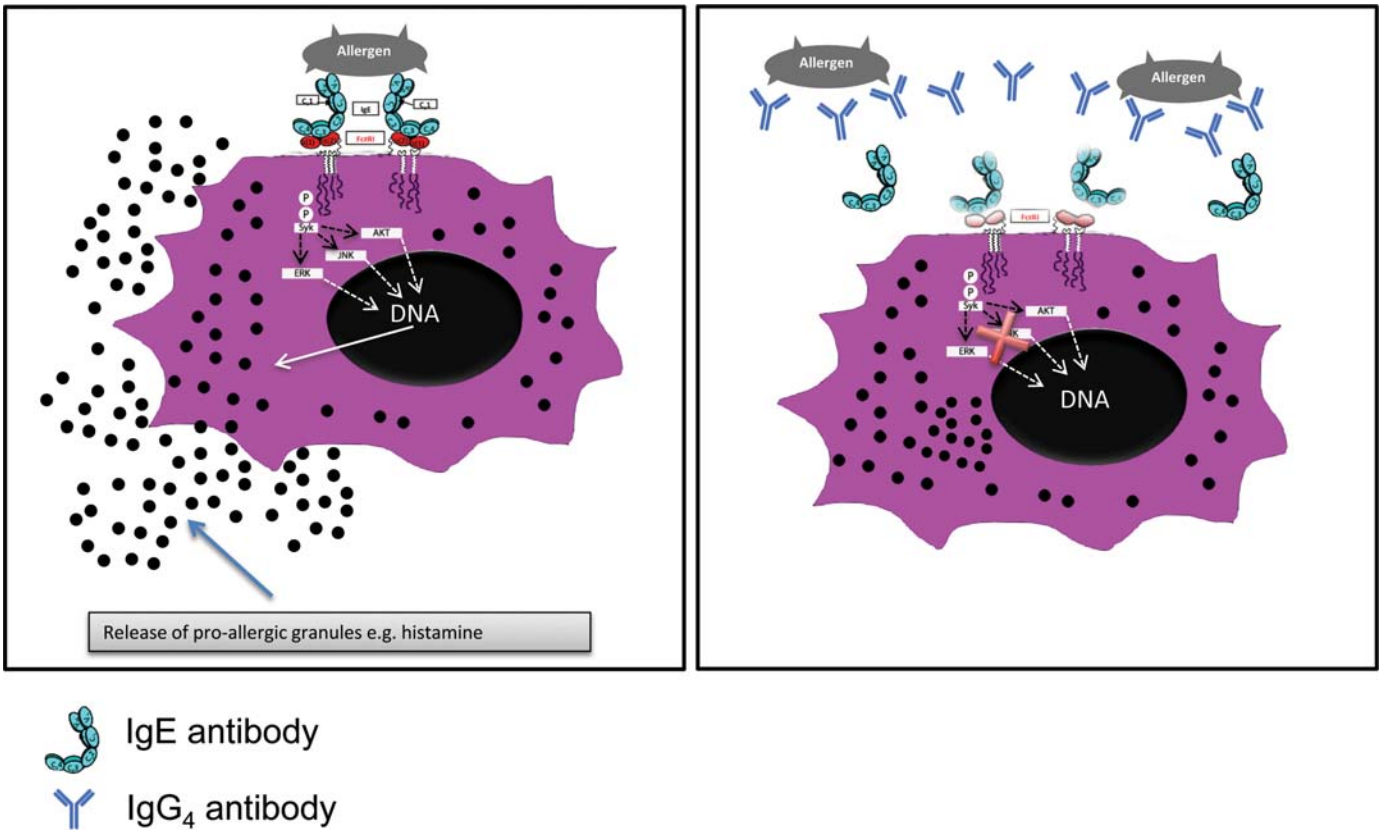

FIGURE 2 IgG4 antibodies induced by allergen immunotherapies modulate IgE-mediated activation of effector cells. The allergic response mediated by multivalent allergen-IgE FcεRI complex assembly and downstream signaling events leading to release of inflammatory mediators (left) may be blocked by adaptive immune responses triggered in response to SIT with recombinant antigens. IgG4 antibodies, induced following SIT, could compete with IgE for binding to allergens and prevent the formation of allergen-IgE-FcεRI complexes on the surface of effector cells, blocking effector functions such as degranulation (right). Syk, spleen tyrosine kinase; AKT, Ak strain thymoma serine/threonine-specific protein kinase; ERK, extracellular signal-regulated kinase; JNK, Jun N-terminal protein kinase; P, phosphorylation. doi:10.1128/microbiolspec.AID-0006-2012.f2

maintained and remain higher than those measured from individuals with diagnosed active cow's milk allergies (134, 135). Finally, favored production of IgG4 antibodies was recently demonstrated in patients with melanomas and extrahepatic cholangiocarcinomas, suggesting the association of IgG4 antibodies with immune tolerance favored by IL-10-rich environments such as those in tumors (136, 137).

THE OPPOSITE SIDE OF THE COIN: HARNESSING THE ALLERGIC RESPONSE AGAINST CANCER AND THE EMERGENCE OF ANTIBODIES OF THE IgE CLASS FOR CANCER THERAPY

Monoclonal antibodies of the IgG class have emerged as an important therapeutic modality over the last 20 years, and a number of these agents are in clinical use for the treatment of cancer. There have been many efforts to enhance the efficacy of these antibodies by engineering Fc regions with enhanced affinity to Fc receptors to create antibodies with desirable pharmacokinetic properties and improved cellular immune functions. Our group's approach has been to enhance antibody Fc-mediated functions by engineering therapeutic antitumoral antibodies with Fc regions of the IgE class (138). Employing this strategy, therapeutic antibodies of the IgE class may confer several advantages over antibodies of the IgG class, particularly with respect to the treatment of solid tumors. The advantages of IgE would include (i) high affinity of IgE to its Fcε receptors; (ii) lack of IgE inhibitory Fc receptors; (iii) natural immune activatory functions in tissues; (iv) presence of IgE effector cells in tumors; (v) the activation of Fc receptors (i.e., FcεRI and FcεRII) other than Fcγ receptors on a set of effector cells (e.g., mast cells, eosinophils, and

monocytes/macrophages) different from those bearing IgG receptors; and (vi) desirable pharmacokinetic properties such as fast clearance from circulation, reducing the chance of antibody-neutralizing antibody responses (35, 139, 140, 141).

Exploiting IgE as a novel class of therapeutic antibodies may prove successful in harnessing a potent allergic response against tumor cells. The multimerization of therapeutic IgE antibodies bound to multimeric antigens or multivalent antigens expressed on tumor cells can result in the cross-linking of antibody receptors on immune cells, high-avidity binding and cell activation, and the release of inflammatory mediators such as histamines, leukotrienes, tryptases, inflammatory cytokines, and cytotoxic granules which would result in tumor cell death. We have demonstrated that IgE antibodies targeting the tumor antigens folate receptor alpha (FRα) and the human epidermal growth factor receptor 2 (HER2/neu) are capable of mediating effective cytotoxicity against tumor cells through IgE Fc-mediated interactions with Fcε receptors present on frequently tumor-resident immune cells such as monocytes, mast cells, and eosinophils (34, 35, 139, 140, 142). Furthermore, in a number of animal models of ovarian carcinoma, the administration of MOv18 IgE antibodies, which target the tumor antigen FRα, resulted in increased survival and restriction of tumor growth compared to dosing with the IgG1 counterparts (35, 139, 140). Thus, IgE antibodies targeting tumor antigens are capable of mediating allergic responses in solid tumors by the activation of immune cells generally involved in allergic responses, and such a strategy resulted in the redirection of the cytotoxic effects of these immune cells against tumor cells (141).

We envisage that a successful clinical utility setting for therapeutic IgE antibodies includes the selection of appropriate disease settings such as those of solid tumor types, with immune cell infiltrates bearing Fcε receptors capable of mediating potent cytotoxic effects. The systemic administration of IgE therapeutic antibodies also warrants immunological monitoring of patients, to ensure allergic responses are primarily harnessed in solid tumors and do not lead to potentially harmful type 1 hypersensitivity responses in patient circulation. Here, since only soluble multimeric antigens at high concentrations in blood would have the potential to cross-link IgE-FcεRI complexes that may give rise to type 1 hypersensitivity, the design of antibodies to antigenic targets known to be shed in multimeric form in the patient circulation should be avoided. Of note, our most advanced IgE class therapeutic antibody candidate, MOv18 IgE, is designed against the cell surface tumor antigen FRα which is shed in low levels and in a monomeric form in human sera. To address concerns of potential type 1 hypersensitivity responses upon administration of antibody in patients, we have evaluated the activation of human immune cells involved in systemic allergic responses, such as basophils and mast cells. We conducted these experiments using two ex vivo and in vitro functional readouts of effector cell activation following the addition of therapeutic IgE antibodies in blood and sera from patients with cancer and healthy volunteers. In both of these readouts, we have found a lack of human immune cell activation, even in the presence of soluble forms of tumor antigens. These findings suggest that this approach bears low risk of systemic type 1 hypersensitivity (143). This is likely due to the absence of IgE cross-linking in blood, even in the presence of soluble tumor antigens and circulating tumor cells at the highest reported physiological amounts in patients. These studies provided support for the potential safety of a tumor antigen-specific IgE antibody administered in the patient's circulation (138, 144).

In conclusion, we propose that therapeutic IgE antibodies may harness allergic responses in individuals with cancer, resulting in significant antitumor effects, and that this antibody class has the potential to emerge as an important therapeutic modality to direct IgE immune responses against cancer. We await the testing of the first therapeutic IgE in humans and continue in the design and development of IgE antibodies for the treatment of many malignancies, including breast cancer, ovarian cancer, and melanoma.

CONCLUDING REMARKS AND FUTURE ROLES OF ANTIBODIES IN THERAPY

Key roles of IgE class antibodies in allergic diseases, in the associated allergic inflammation, and in parasitic and infectious diseases have emerged over 4 decades of research. Increased levels of IgE antibodies specific to allergens are central to the pathogenesis, exacerbation, and diagnosis of many allergic conditions. Increased levels of IgE are also associated with sensitivity to infections in patients with HIES, while certain viral infections also trigger class switching and production of IgE that can lead to symptom exacerbations in allergic individuals. On the other hand, IgE antibodies are also thought to confer protective roles in parasitic infections. Potential functions for the IgG class are also reported in some anaphylactic responses, while IgG4 subclass and IgA antibodies are associated with development of

natural and specific allergen immunotherapy-induced tolerance to allergen exposure. These provide support for the important contributions of antibodies in allergic and infectious diseases.

Importantly, the approval of omalizumab for the treatment of a number of IgE-mediated allergies constituted a proof-of-principle agent and a significant milestone in the clinical management of patients. Omalizumab has also contributed new understanding of the interactions between IgE, its receptors, and allergens and novel insights into the mechanisms by which these interactions impact on the development of allergic inflammation. These developments have also placed these interactions at the forefront of translational efforts to design novel treatments. Furthermore, they have prompted increased focus on the intelligent design of new more effective therapeutics with improved functional properties or on the design of agents with multimodal actions, all aiming at achieving better efficacy than that shown with application of omalizumab in the clinic. Such strategies would include monoclonal antibodies but also small molecules with the capacity to interfere with IgE-FcεR interactions (145).

The significance of antibodies in the treatment of allergic diseases has also been suggested in a different context, through the pivotal findings that allergen immunotherapy triggers increased levels of protective humoral responses in patients. These responses manifest in the form of IgG4 subclass antibodies. All these developments highlight the central role of antibodies in passive but also active immunotherapy of allergic diseases. Furthermore, the mechanisms by which IgG4 antibodies operate are currently an area of intense research activity and may themselves lead to the rational design of active immunotherapies designed to trigger more effective humoral responses capable of regulating IgE-mediated inflammation. Another potential future strategy may be the design of engineered IgG4 subclass antibody therapeutics capable of passively blocking IgE-mediated responses in patients with allergies.

Finally, IgE antibodies and their potent effector functions, known to confer protection in parasitic infections and to contribute to allergic diseases, may be utilized as therapeutics for cancer. A number of groups have examined the concept of raising IgE antibodies through a number of active immunization approaches and also by harnessing powerful immune effector cell functions against cancer cells through passive immunotherapy with monoclonal antibodies (138, 139, 140, 142, 146, 147, 148, 149, 150, 151, 152, 153). A growing number of studies, including those from our group, demonstrate the promise of activating allergic responses against cancer and have defined the emerging field of AllergoOncology (138, 154, 155). Passive immunotherapy with engineered antibodies has, in our hands, demonstrated considerable efficacy with a number of antibody therapeutic candidates, in different oncological disease settings, and in a number of disease-relevant models (138). Ultimately, the utility of an antibody class such as IgE, commonly associated with the pathogenesis of allergies, may find a new purpose in cancer therapy. Our planned first-in-class clinical study of our anti-FRα IgE antibody will undoubtedly provide new insights into the hypothesis that the potent effector activatory functions of IgE antibodies could be directed against malignancy.

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