

IgE and immunoglobulin free light chains in allergic disease: New therapeutic opportunities

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The prevalence of allergy and associated diseases has dramatically increased during the last few decades. These hypersensitivity reactions can be subdivided into IgE- and non-IgE-dependent diseases. Anti-IgE approaches, such as omalizumab, have provided a novel treatment for allergic diseases, and have demonstrated benefit in severe allergic asthma and seasonal allergies. Nevertheless, this approach does not eliminate disease and many patients do not respond to treatment. The newly described mechanism for allergic diseases based on mast cell sensitization by Ig free light chains is exciting and may provide another approach to the treatment of allergic diseases. The peptide inhibitor F-991 has demonstrated remarkable biological activity in a number of animal models of allergic diseases, and promise for the potential treatment of allergic diseases in humans.

Keywords Allergy, F-991, hypersensitivity, IgE, Ig free light chain, mast cell, non-IgE, omalizumab

Introduction

The prevalence of allergy and associated diseases has dramatically increased during the last few decades. Allergic reactions are caused by an overreaction of the immune system to usually harmless foreign antigens. It is estimated that between 10 to 30% of the population in developed western countries is likely to suffer from clinical symptoms of allergy [1-3]. Many factors have been proposed as potential contributors to allergic sensitization, which include genetic factors, increased hygiene, viral infections, air pollution and diet [2,4-6].

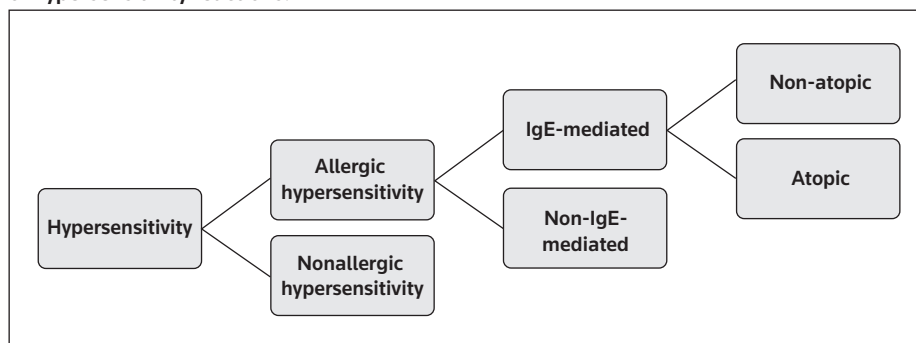
IgE versus non-IgE-mediated allergic diseases

In a position paper by the European Academy of Allergology and Clinical Immunology task force, the nomenclature for allergic and related reactions has been defined based on the present knowledge of the pathomechanism of allergic diseases [7,8]. This definition can be used independently of the target organ or patient age group and is based on the mechanisms involved in initiating and mediating allergic responses (Figure 1). All allergic responses can be grouped as hypersensitivity responses. Hypersensitivity responses with an underlying immunological mechanism are defined as allergic, while those without a 'suspected' role of the immune system are grouped as 'nonallergic hypersensitivity'. Allergic hypersensitivity can be subdivided further to IgE- and non-IgE-mediated allergies. IgE-mediated allergies can

be classified as non-atopic and atopic. Atopy refers to the personal and/or familial tendency to become sensitized to produce IgE antibodies in response to ordinary exposures to allergens, usually proteins [7,8].

IgE is considered an important mediator in the pathogenesis of allergic diseases, such as asthma [9]. Strong IgE antibody responses in early life to, for example, constituents in cow's milk and hen's eggs, are markers for atopic reactivity that result in food allergy/intolerance. Later in life, these individuals may develop sensitization to aeroallergens, which may lead to the development of other clinical symptoms, such as eczema, rhinitis or asthma. This progress of allergic clinical manifestations over a lifetime is often referred to as the 'allergic march' [10-13]. Conversely, a considerable number of patients present with allergic symptoms without a clear causal factor, such as increased total or specific IgE or a positive skin prick test [14]. For example, > 200 million patients worldwide suffer from non-allergic rhinitis and approximately 40% of patients with asthma have no clear signs of the involvement of IgE-mediated mechanisms [15]. Atopic eczema (also known as atopic dermatitis) is a chronic relapsing inflammatory skin disease, and affects 10 to 20% of children and 1 to 3% of adults. It has been estimated that the nonallergic variant of eczema accounts for up to 30% of total eczema cases [16,17].

By definition, an immunological mechanism is involved in the pathogenesis of all allergic hypersensitivity responses,

Figure 1. Classification of hypersensitivity reactions.

Hypersensitivity reactions can be subdivided into allergic and nonallergic hypersensitivity. In allergic hypersensitivity responses, an immunological mechanism is defined or strongly suspected, while in nonallergic hypersensitivity, immunological mechanisms are excluded or cannot be proven, for example, in aspirin hypersensitivity. Allergic hypersensitivity can be caused by IgE- and non-IgE-mediated mechanisms. Non-IgE-mediated mechanisms include cellular and Ig-driven allergic responses. IgE-mediated allergies can be classified as non-atopic and atopic. Atopy is considered a familial and personal tendency to develop allergen-specific IgE on exposure to environmental allergens, and typical allergic symptoms result.

though further mechanistic studies are needed to fully understand the basic mechanisms of both IgE- and non-IgE-mediated allergies. In this review, the possibilities of therapeutically interfering in IgE- and non-IgE-mediated allergies are discussed. Furthermore, the role of a novel pathway to elicit antigen-specific non-IgE-mediated hypersensitivity responses is outlined.

Ig free light chain-mediated allergic responses

Plasma cells produce and secrete different classes of Igs, such as IgM, IgG, IgA, IgD and IgE (Figure 2). The antibody class switching occurs in mature B-cells, and is dependent on antigen stimulation and costimulatory signals. During synthesis of Igs, heavy chains and Ig light chains are combined. Either κ or λ Ig light chains are used during the assembly of complete Igs. In humans, there is a balanced use of κ and λ light chains for Ig synthesis, while in mice, for example, there is a predominant use (> 95%) of κ light chains [18]. Also, a considerable amount of Ig light chains is secreted by plasma cells in its free form. They exist as monomers (molecular weight 22 to 27 kDa), and covalently and non-covalently bound dimers (molecular weight 44 to 55 kDa) [19]. Polyclonal free light chains are present in many biological fluids, including serum, urine, cerebrospinal and synovial fluid, in healthy individuals. A wide range of 'normal' values for levels of free κ (1.2 to 21 mg/l) and free λ light chains (3.0 to 42 mg/l) in serum have been reported [19-21]. Ig free light chain concentrations are increased in patients with allergic diseases, such as asthma [22], rhinitis [D Powe, T Groot Kormelink, M Sisson, B Blokhuis, M Kramer, N Jones & F Redegeld, unpublished data] and atopic dermatitis [23]. In addition, numerous autoimmune diseases are associated with increased Ig free light chains in serum or other compartments [19,21,24], while several studies have demonstrated that increased concentrations of free light chains correlate with disease activity [25,26].

Although Ig free light chains can exert various enzymatic activities [21], one biological property of Ig free light

chains has been described that may be of particular interest in the pathogenesis of chronic inflammatory diseases. In previous years, Ig free light chains have been shown to elicit immediate hypersensitivity-like responses [27]. Ig free light chain can also bind to mast cells, and crosslinking of surface-bound light chains leads to mast cell activation and initiation of an inflammatory response. Passive transfer of antigen-specific free light chains into naive mice followed by challenge with cognate antigen resulted in skin, airway or intestinal inflammation, depending on the site of challenge [22,27].

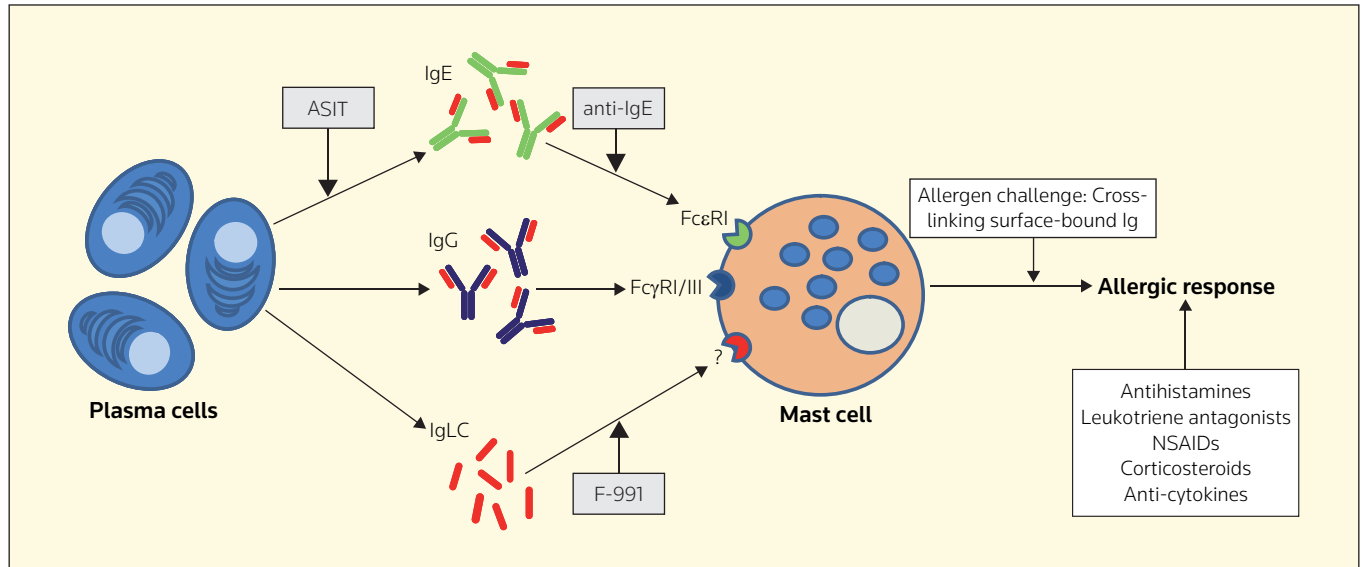
Treatment of non-IgE-mediated hypersensitivity with F-991

F-991 (Clinquest Group) is a specific immunoglobulin free light chain antagonist. It is an N-acetylated and C-amidated 9mer peptide whose sequence is derived from Tamm-Horsfall protein, a major urinary protein. In previous studies, Tamm-Horsfall protein bound to Ig free light chains [28]. F-991 binds to Ig free light chains and prevents sensitization of tissue-resident mast cells. F-991 does not interfere with hypersensitivity reactions elicited by IgE or IgG1 [27]. The efficacy of F-991 has been evaluated in diverse preclinical models of inflammatory diseases. Furthermore, the safety of F-991 has been determined in toxicological studies and in healthy individuals, and in a small-scale study the clinical efficacy of F-991 has been tested in house dust mite (HDM)- and peanut (PN)-allergic patients.

Preclinical studies

Immediate hypersensitivity-like responses

Intravenous injection of hapten-specific Ig free light chains into mice, followed by local allergen challenge leads to mast cell activation and an inflammatory response [22,27]. For example, intranasal allergen challenge was followed by an acute bronchoconstriction and plasma extravasation [22]. F-991 specifically inhibited Ig free light chain-induced inflammatory responses, while no effect on, for example, IgE- or IgG-induced hypersensitivity

Figure 2. Schematic representation of the induction of allergic hypersensitivity via IgE- and non-IgE-mediated mechanisms.

IgE, IgG and Ig free light chains (IgLCs) generated by plasma cells sensitize mast cells via specific receptors. Upon second encounter with the allergen/antigen, cell-surface bound Igs are crosslinked, resulting in mast cell activation and the release of proinflammatory mediators. At several stages during this allergic cascade, therapeutic intervention is possible. Allergen-specific immunotherapy (**ASIT**) is used to induce tolerance to specific allergens and is currently the only method to prevent allergic sensitization. Binding of IgE or IgLCs to mast cells can be prevented by anti-IgE (eg, omalizumab) or F-991 (Clinquest Group), respectively. Downstream, biological effects of mast cell-derived proinflammatory mediators can be reduced by various drugs, such as antihistamines, leukotriene (receptor) antagonists, NSAIDs, corticosteroids and anti-cytokines.

responses was observed [27]. F-991 can be administered via various routes.

Contact hypersensitivity

Hapten-induced contact hypersensitivity responses in mice can be effectively inhibited by prophylactic local or systemic administration of F-991. Local edema formation directly after challenge and late-phase inflammation was greatly inhibited by F-991. Also, therapeutic intervention with F-991 (2 h after hapten challenge) significantly reduced late-phase skin inflammation [27,29].

Asthma

In a murine model of non-IgE mediated asthma, administration of F-991 at the time of allergen provocation prevented clinical signs of asthma [30,31], such as the development of airway hyper-responsiveness and inflammation [22].

Inflammatory bowel disease

Clinical symptoms of inflammatory bowel disease induced after rectal challenge in dinitrofluorobenzene-sensitized mice were greatly reduced after systemic administration of F-991 at the time of allergen provocation [32], [A Rijnerse, F Redegeld, B Blokhuis, M van der Heijden, A Te Velde, I Pronk, D Hommes, F Nijkamp, A Koster, A Kraneveld, unpublished data].

Food allergy

In mouse models of cow's milk allergy, either increased IgE or Ig free light chain levels can be detected, depending on the food allergen or sensitization conditions

applied [33]. Nevertheless, mice displayed similar allergic symptoms, providing evidence that Ig free light chain could act as an alternative to IgE to elicit these responses. F-991 inhibited hypersensitivity responses that were produced under sensitization conditions that cause an enhanced production of free light chains in models of cow's milk allergy [B Schouten, E van Esch, B Blokhuis, G Hofman, L Knippels, L Willemsen, F Redegeld, J Garssen, unpublished data].

In summary, F-991 has been demonstrated to exert potent biological activity in diverse preclinical models of disease. Further experiments to explore the role of free light chains and the therapeutic potential of F-991 in other preclinical models of disease are currently underway [T Groot Kormelink, M Thio, F Redegeld, unpublished data].

Clinical studies

Based upon the promising therapeutic effects in the preclinical models of allergic disease, the potential clinical efficacy of F-991 for human allergic disorders was investigated. A phase I clinical trial demonstrated that F-991 has no adverse effects in toxicological studies or when administered intravenously to healthy individuals [34]. In a proof-of-principle study, the effect of prophylactic, local administration of F-991 on allergen-induced skin reactions in HDM- and PN-allergic patients was investigated. In these studies, F-991 was administered by local intracutaneous injection at the site of allergen challenge. Both wheal size and corresponding erythema were measured at 15 min and 6 h after

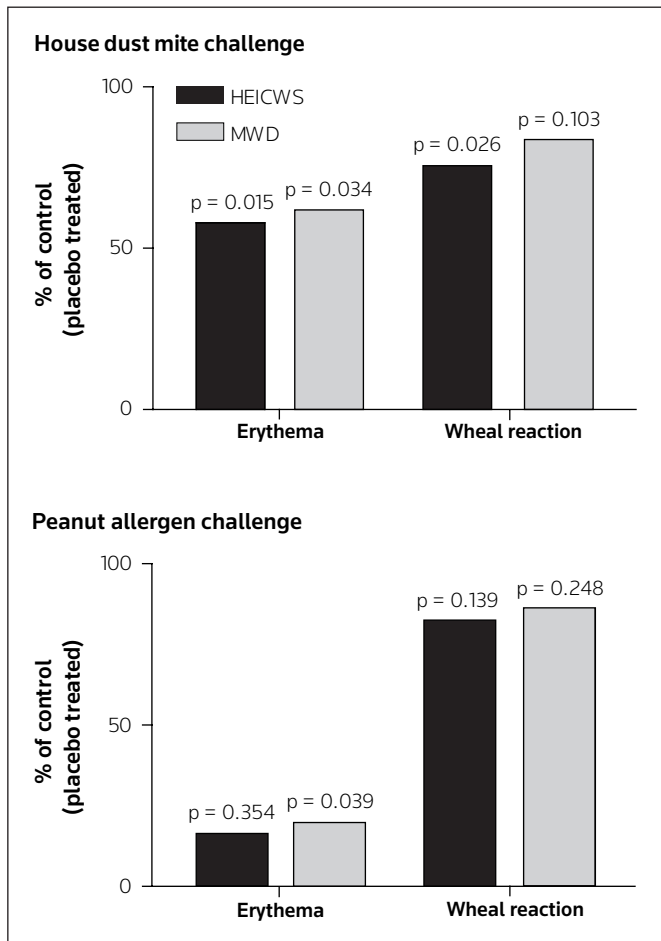
injection of the allergen extract. HDM- and PN-allergic individuals ($n = 24$ and 15 , respectively) were included in this study [F Roossien, Fornix BioSciences NV, personal communication]. Unfortunately, the interpretation of the results of this study was complicated by the induction of a non-specific response toward the formulated F-991 at the site of injection. However, when the immunological allergic effect of allergen challenge was corrected for this F-991-induced irritant reaction (*post-hoc* analysis), a significantly smaller early-phase skin reaction after treatment with F-991 compared with placebo was observed in HDM-allergic patients, suggesting clinical efficacy of F-991 in the treatment of these allergies (Figure 3). Proof-of-concept studies using other routes of administration are warranted to further determine the role of IgLC and the clinical efficacy of F-991 in allergic diseases. The planning of such studies, which will be conducted by Clinquest Pharmaceutical Innovations BV, is underway [R Jan Rutten and C Wortel, personal communication].

Treatment of allergic hypersensitivity with anti-IgE

Most allergic diseases (eg, allergic asthma, allergic rhinitis and food allergies) are mediated by IgE. IgE binds to receptors on the surface of inflammatory cells, most importantly mast cells and basophils, which contain and produce a number of inflammatory mediators. When the cell-bound IgE binds allergen, it causes activation of mast cells and basophils to release pre-formed inflammatory mediators, such as histamine, and to produce additional proinflammatory products. Thus, one strategy for therapeutically treating allergic diseases is to prevent IgE from binding to inflammatory cells [35]. This is the concept behind omalizumab, which is a humanized mAb that binds to a specific region of IgE and prevents it from binding to the cellular IgE receptor. Because omalizumab binds to only free soluble IgE, it essentially removes free IgE from the circulation, thereby preventing the 'arming' of inflammatory cells [35]. Because the inflammatory cells can no longer respond to allergens, the allergic symptoms are reduced. To achieve this, almost all free IgE must be removed because even a small amount of IgE will cause an allergic response. Of interest, upon cessation of omalizumab treatment, even after 6 years of continuous treatment, free IgE levels return, as does the allergic disease [36].

Anti-IgE has been tested in > 20 completed clinical trials of allergic asthma, allergic rhinitis/seasonal allergies, food allergies (peanut), anti-IgE therapies co-administered with specific immunotherapies (rush or subcutaneous immunotherapy), and patients with environmental allergies, such as latex and bee sting allergies [1,37,38]. A meta-analysis of seven clinical trials in severe persistent asthma demonstrated that omalizumab significantly reduced the number of asthma exacerbations, the number of emergency medical visits, the use of inhaled steroids, and improved quality-of-life and lung function; however, not all treated patients responded to omalizumab

Figure 3. Effect of F-991 on house dust mite- and peanut-induced skin reactions.



Wheal reaction and erythema were measured as mean wheal diameter (MWD) or histamine equivalent intracutaneous wheal size (HEICWS) at 15 min after local injection of allergen in the presence or absence of F-991. Values for skin reaction were corrected for the significant irritant effect of F-991.

treatment, and those who responded the best were the most severe asthma patients who were also using high-dose inhaled corticosteroids and had poor lung function [38]. By examining several disease parameters that comprise the composite definition of response to omalizumab, the response range with omalizumab treatment was 60 to 67% of treated patients compared with a response rate in patients receiving placebo of 40 to 55% [39]. The reasons for omalizumab unresponsiveness are unknown, but could include the observation that as the disease progresses there are direct effects on other inflammatory cells to perpetuate the asthma symptoms that do not require IgE-mediated triggering. This could include non-IgE-mediated mast cell activation, direct allergen effects on inflammatory T-cells, autoantibodies to the IgE receptor and bronchial hyperreactivity, among other possibilities. Based on these results, anti-IgE treatment has been approved for use in patients over 12 years of age with moderate-to-severe allergic asthma in the US, Europe and a number of other countries. FDA guidelines

for asthma treatment do not recommend omalizumab as a first-line treatment for asthma, but recommend it as adjunctive treatment for patients with severe uncontrolled disease who fail to respond to inhaled corticosteroids and long-acting β -agonist therapies [40].

Omalizumab has also been clinically tested in several clinical trials for allergic rhinitis (seasonal allergies) in which it demonstrated benefit in significantly reducing the nasal severity score, the use of antihistamines, and improving quality-of-life [41]. Omalizumab was also beneficial in trials examining this drug in combination with specific allergen immunotherapy [42]. One trial of TNX-901, an anti-IgE mAb, in patients with food allergies demonstrated that anti-IgE reduced the sensitivity of PN-allergy patients to challenge with a small dose of peanut flour [43,44]. The development of TNX-901 has since been discontinued, although the reason for this is unknown [45]. Anti-IgE mAb therapy is not currently approved for use in allergic rhinitis or peanut allergies.

In all clinical trials to date, omalizumab has demonstrated an excellent safety profile [38]. There have been no severe side effects and anti-omalizumab antibody responses are rare in treated patients. There have been no increases in parasitic infections and a report that there may be an increased incidence of cancer in omalizumab-treated patients may not be due to treatment. There have been a few reports of severe anaphylactic responses in treated patients (0.2%). It is unknown if these are responses to omalizumab itself, or to an excipient in the formulation [44]. Nevertheless, the FDA required a warning be added to the omalizumab label regarding the potential for anaphylactic responses.

Despite the success of omalizumab in treating allergic diseases, several issues remain. First, it is not a panacea and is not effective in all patients. In its current formulation, there are dosing limitations, and it can only be administered in a clinical setting. Importantly, omalizumab is also expensive. Cost-benefit analyses suggest treatment at its current price is only positive for the most severe steroid-/ β -agonist-resistant asthma patients [46]. This precludes use in less severe non-life-threatening diseases, such as allergic rhinitis. To address some of these issues, efforts are underway to improve the solubility of the omalizumab formulation, which may improve the potential for home delivery, among other things. In other approaches to block IgE binding to its receptor, mAbs, including an anti-Fc ϵ RI α mAb known as 15/1 mAb, that bind to the IgE receptor may be as effective as anti-IgE therapy [47]. Also, a vaccine to block IgE antibodies demonstrated similar properties to omalizumab and could be used to vaccinate patients to reduce allergic responses [48].

The removal of free IgE by anti-IgE treatment is not beneficial for some patients with allergic asthma, which suggests that, in part, the disease mechanism in allergic asthma may involve biological mechanisms that do not

involve IgE in up to 40% of asthma patients [49]. In this regard, there is a growing body of data suggesting that allergen-specific free Ig light chains can sensitize mast cells and, when exposed to allergen, can mediate mast cell activation and the onset of allergic diseases [19,21,22,27]. This in essence is a mechanism for allergic diseases that does not directly involve IgE and could play a role in allergic responses in patients who do not respond to omalizumab. Importantly, in animal models, treatment with F-991, which prevents free light chain sensitization of mast cells, has demonstrated efficacy in reducing symptoms of several allergic diseases [22,27,29]. As F-991 continues clinical testing, it will be important to determine if this inhibitor will be of benefit in treating patients with allergic asthma who fail to respond to omalizumab. In addition, F-991 has several other advantages over omalizumab, including cost of production (F-991 is a small synthetic peptide), formulation, ease of administration and the potential to treat a broader spectrum of allergic diseases.

Conclusion

In summary, anti-IgE approaches, such as omalizumab, have provided a novel treatment for allergic diseases, and have demonstrated benefits in severe allergic asthma and seasonal allergies. Nevertheless, this approach does not eliminate disease and many patients do not respond to treatment. The newly described mechanism for allergic diseases based on mast cell sensitization by Ig free light chains is exciting and may provide another approach to treatment. The peptide inhibitor F-991 has demonstrated remarkable biological activity in a number of animal models of allergic diseases and has promise for the potential treatment of those diseases in humans.

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