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The Major Cat Allergen, Fel d 1, in Diagnosis and Therapy

Hans Grönlund Tiiu Saarne Guro Gafvelin Marianne van Hage

Clinical Immunology and Allergy Unit, Department of Medicine, Karolinska Institutet and University Hospital, Stockholm, Sweden

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Abstract
Sensitization to cat is a common cause of allergic disease all over the world. Symptoms range from mild rhinoconjunctivitis to potentially life-threatening asthmatic exacerbations. In vivo and in vitro diagnostics of cat allergy is currently based on cat dander extract. As allergen extracts from natural sources are heterogeneous in composition, the allergen content may vary. With the introduction of allergens produced by recombinant techniques, a large panel of recombinant allergenic molecules including the major cat allergen, recombinant Fel d 1, has become available for immunological investigations, diagnosis and treatment. Studies have shown that this single allergen, which belongs to the uteroglobin protein family, is at least as good as cat dander extract in identifying cat-allergic patients. The introduction of recombinant Fel d 1-based tests into clinical practice will increase our knowledge of this single allergen molecule as a diagnostic tool and improve the selection for therapy of cat allergy. Several different modes for allergen-specific immunotherapy of cat allergy based on Fel d 1 have been developed. These include Fel d 1 hypoallergens and allergen constructs where Fel d 1 is coupled to immunomodulatory proteins or carriers. The approaches have been evaluated in experimental in vitro and in vivo model systems with promising results. In addition, immunotherapy with Fel d 1 peptides containing T-cell epitopes has been tested in clinical trials. After initial problems with adverse reactions, more recent data show that peptide immunotherapy modulates the immune response to Fel d 1 and reduces early- and late-phase effector reactions in cat-allergic patients.

Introduction

Cat allergens are among the most important indoor allergens and a common cause of IgE-mediated allergic disease world-wide [1, 2]. The prevalence of cat sensitization is 10–15% among adults [3–5]. The symptoms range from rhinoconjunctivitis to potentially life-threatening asthmatic exacerbations. Sensitization in childhood is a predictor for subsequent rhinoconjunctivitis and asthma [6, 7]. Studies have shown that sensitization to cat is a strong risk factor for asthma [8, 9]. In order to prevent respiratory allergic disease, early detection of sensitization is useful.

Cat Allergens

Attempts to characterize the allergenic content of cat dander and hair have so far indicated that less than 10 components may bind IgE [10, 11]. Of these, 5 are well documented [12]. Beside the most important and potent

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Correspondence to: Dr. Hans Grönlund
Department of Medicine, Clinical Immunology and Allergy Unit
Karolinska Institutet and University Hospital
Solna L2:04, SE–171 76 Stockholm (Sweden)
Tel. +46 8 517 766 97, Fax +46 8 33 57 24, E-Mail hans.gronlund@ki.se

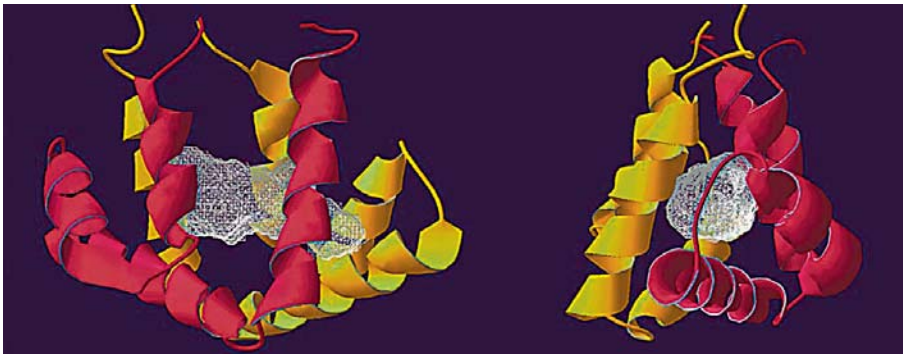

Fig. 1. Ribbon representation of the 3-dimensional structure of Fel d 1 and the cavity (white) within the protein. The protein is displayed from 2 different orientations, with a rotation of about 90° around the vertical axis. The helices corresponding to chains 2 and 1 are given in red and yellow, respectively. From Kaiser et al. [35].

of the allergens, the secretoglobin Fel d 1 (originally termed 'Cat 1') [13], cat dander also contains several cross-reactive allergens of less impact. Despite being a dominant protein in dander, the 67-kDa acidic protein cat albumin, Fel d 2 [14–16], reacts with IgE from 20 to 35% of cat-sensitized subjects [17]. Among other allergens found in dander is the evolutionary conserved cysteine protease inhibitor allergen cystatin, Fel d 3, recognized by at least 10% of patients [18], and the 20-kDa lipocalin allergen, Fel d 4, which is reported for approximately 60% of cat-allergic individuals, although typically with low IgE levels [19]. The latest addition to the listed allergens is the 400-kDa cat immunoglobulin A, Fel d 5, with IgE antibodies reported in nearly 40% of patients [20]. Also cat IgM, Fel d 6, has been suggested as an allergen; however, this 800- to 1,000-kDa antibody is less well documented [20]. The particular feature of these 2 allergens is that the very dominant IgE-binding epitopes are localized to the galactose-α-1,3-galactose carbohydrate structure on the heavy chain of these antibodies [21].

Biochemical Characterization of Fel d 1

Fel d 1, a protein with unknown function, is produced by the skin and by salivary and lacrimal glands of the cat [10, 22–27]. Fel d 1 is transferred to the pelt by licking and grooming [28, 29]. Dried saliva and dandruff are spread from the cat hair as small airborne particles into the surrounding environment, where they may cause sensitization in susceptible individuals.

Over the years, Fel d 1 has been extensively characterized, both by biochemical and immunological methods such as CD spectrum [30] and amino acid sequencing [31], cloning [32], chrystallography [33–35], analysis of T-cell epitope repertoires [36, 37], serological measurements of patients' IgE antibodies [17, 38], as well as epitope mapping by monoclonal antibodies [39–41].

The acidic Fel d 1 glycoprotein [42] appears as 35–39 kDa by size exclusion chromatography [31], including 10–20% complex N-linked tri-antennary carbohydrate structure [31, 42]. The molecular size of Fel d 1 suggests that it forms tetramers consisting of two 18-kDa non-covalently linked heterodimers [31, 42]. Each dimer contains 2 antiparallel polypeptides, one 70 amino acid residue and 8-kDa chain 1 and one 10-kDa chain 2 [32, 43], where the glycan moiety of the protein is located to chain 2. The 2 chains are covalently linked by 3 inter-chain disulphide bonds [42]. The primary structures of Fel d 1 were described in detail when cloned in 1991 [32]. The 2 polypeptide chains are co-expressed in both skin and salivary glands, where a slightly shorter version (90 amino acids) of chain 2 preferentially can be found in skin, whereas a longer version (92 amino acids) is expressed in salivary glands [44]. Truncations and several isoforms residing both in chain 1 and chain 2 have been described [42, 44].

The first indication that Fel d 1 belongs to the uteroglobin protein family came from studies of sequence similarities between chain 1 and other family members [45] showing 30% sequence identity. However, the question was not resolved. Because of the low sequence identity between chain 2 and other uteroglobins, a barely detectable 10–15% homology was identified. The structure of Fel d 1 was solved in 2003 [33] (fig. 1). The structure revealed an all-helical protein containing 8 helices. The 2 Fel d 1 polypeptides were very similar in structure, despite only 10–15% sequence identity. Fel d 1 and orthologues of the uteroglobin protein family, such as rabbit uteroglobin, the human Clara cell protein, CC16, and the mouse androgen-binding protein all include a cavity, which may harbour small ligands [43, 46–48]. Recently, the quaternary structure of Fel d 1 was reported, highlighting 2 new

structural aspects [34]. First, it was shown that the close molecular interaction by the dimer formation causes a profound alteration of the 2 pockets, creating a smaller 350- and a larger 750-Å^3 compartment. The latter is caused by the dislocation of 2 amino acids from the inner lining of the pocket to solvent exposure, thereby creating an entrance to the cavity. Secondly, 3 well-defined Ca^{2+}-binding sites were identified in the Fel d 1 tetramer, a feature also suggested by other uteroglobin structures.

Work by Chapman and Aalberse [49–52] in 1993–1994 aimed at defining B-cell epitopes on Fel d 1 using various preparations of native Fel d 1, overlapping synthetic peptides, monoclonal antibodies and patient sera. In a minority of patients tested, IgE binding was shown by 3 of 21 approximately 15-mer peptides; however, with typically low levels [51]. The result implicated the importance of conformational epitopes on the intact molecule, which is in line with other studies [53]. So far, information about major IgE-binding sites of Fel d 1 is lacking. Several studies have attempted to identify dominant Fel d 1 T-cell epitopes [36] in order to construct peptide-based vaccines [37, 54–56]. Counsell et al. [54] used T-cell lines from 53 cat-allergic subjects and mapped the T-cell reactivity to overlapping peptides spanning chain 1 and chain 2 of Fel d 1. T-cell reactivity was detected to peptides corresponding to the entire sequence of both chains but the most frequent responses were mapped to chain 1. From these data, 2 peptides containing major T-cell epitopes could be identified consisting of amino acid residues 7–33 and 29–55, subsequently applied in clinical trials (see below). The T-cell responses elicited by Fel d 1 peptides have been shown to vary in terms of the cytokine profile between different patient groups and healthy non-allergic subjects [56–58]. Differences in study populations, peptides used for mapping and read-out systems for determining T-cell reactivity obviously complicate the interpretation of T-cell epitope mapping data. However, taken together, T-cell epitopes have been identified in almost all parts of the Fel d 1 protein but most studies support that they are predominantly localized on chain 1.

Fel d 1 in the Diagnosis of Cat Allergy

Current diagnostics of cat allergy using crude cat dander extracts are well established. These extracts contain a variety of allergenic and non-allergenic components and may be difficult to standardize. As mentioned above, characterization of cat dander extract has so far identified 6 allergenic molecules but the majority of patients, 80–95%, have IgE antibodies directed against Fel d 1 [17, 22, 38]. The dominance of Fel d 1 is also emphasized by the finding that more than 60% of all IgE antibodies induced by cat dander are directed to this particular allergen [23]. Cat allergy is unique among allergies to mammals in that the major cat allergen, Fel d 1, is an uteroglobin-like protein and not a lipocalin [33].

In the diagnosis of cat allergy, the possibility of replacing cat dander extract with 1 single allergen, Fel d 1, has been investigated. Ohman and Lowell [22] have in the past studied the IgE antibody reactivity to cat allergens in an allergic population. They reported that 65% of cat-allergic patients were sensitized to cat allergen 1 (Fel d 1). More recently, van Ree and colleagues [17] investigated 509 sera selected on the basis of a positive RAST to cat dander extract. They found that more than 96% of the sera were identified by a not fully refolded mix of the 2 Fel d 1 chains. The negative sera were in general low responders to cat extract. In addition, they demonstrated that the IgE reactivity to Fel d 1 accounts for 88% of the total IgE response to cat allergens. Ichikawa and colleagues [1] assessed the frequency of cat-specific IgE responses to cat dander extract and Fel d 1 among young Japanese patients with asthma who completed a questionnaire on environmental exposure. A total of 161 ImmunoCAP-positive sera were used to compare IgE antibodies to cat dander with IgE to Fel d 1. The authors found that 95% of positive sera (class $\geq$1) had Fel d 1-specific IgE. They concluded that Fel d 1 is an excellent marker for cat sensitization.

We have recently evaluated antibody responses to a properly folded recombinant Fel d 1 (rFel d 1) [38]. Sera from 140 cat-allergic children and adults from Sweden and Austria, suffering from asthma and/or rhinoconjunctivitis, were tested for IgE and IgG4 antibodies to cat dander extract and rFel d 1 by ImmunoCAP. rFel d 1 was covalently bound to the solid support (MIAB, Uppsala, Sweden). Positive IgE antibody responses to rFel d 1 were observed in 95.6% (65 out of 68) of cat-allergic children and in 94.4% (68 out of 72) of adults. Similarly, 64 children and 71 adults were positive by cat dander extract ImmunoCAP. When children from each country were analysed separately or combined, we found that they displayed significantly higher median IgE levels in both rFel d 1 and cat dander extract compared with the group of 72 cat-allergic adult patients (fig. 2). Furthermore, we observed that the IgE levels in rFel d 1 among children with asthma were significantly higher compared to children with rhinoconjunctivitis and adults with asthma (fig. 3). We also analysed the concentration of naturally occurring IgG4

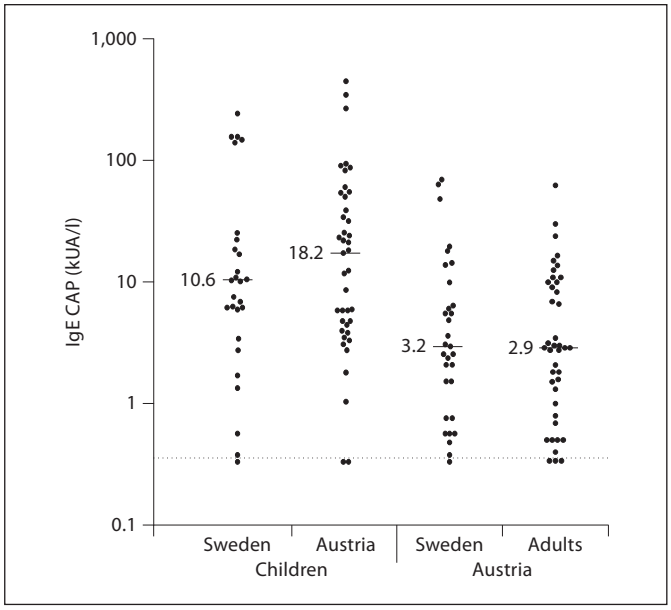

Fig. 2. IgE responses to rFel d 1 in cat-allergic children from Sweden (n = 27) and Austria (n = 41) and adults from Sweden (n = 31) and Austria (n = 41) analysed by ImmunoCAP System. The hatched bar denotes the 0.35 kUA/l cut-off. From Grönlund et al. [38], reprinted with permission from the publisher.

antibodies to rFel d 1. In contrast to IgE, no differences in the IgG4 levels to rFel d 1 were detected between the children and the adult patient groups. However, children with asthma displayed higher IgG4 levels than the asthmatic adults. Thus, a properly folded rFel d 1 is at least as sensitive for in vitro diagnostics of cat allergy as current extract-based tests. Furthermore, increased Fel d 1-specific IgE levels may be a potential risk factor for allergic asthma in children. Fel d 1-based tests are now available and, together with the other cat allergens, they will improve diagnosis and selection of therapy for cat allergy.

Cross-Reactivity of Fel d 1

The association between cat and dog allergy has raised the question whether this phenomenon is due to co-sensitization to different allergenic molecules or whether cross-reactivity with allergens present in cat and dog dander may also play an important role. Results provide evidence that cat and dog dander, besides Fel d 2/Can f 3 (albumin) [15, 59, 60] and perhaps Fel d 4 (lipocalin) [19], contain other cross-reactive antigens. Reininger et al. [61] have examined whether dog dander extract contains an allergen that cross-reacts with the major cat allergen, Fel d 1. Sera from 36 allergic patients containing IgE antibodies to cat and dog dander extracts were examined in quantitative ImmunoCAP inhibition studies with rFel d 1. IgE immunoblot inhibition experiments were performed, and a specific rabbit antiserum was raised against rFel d 1 to search for a cross-reactive allergen in dog dander extracts from several different dog breeds. The authors found that in 25% of 36 Fel d 1-reactive cat-allergic patients, more than 50% inhibition of IgE reactivity to dog allergens was achieved with recombinant Fel d 1. A Fel d 1 cross-reactive 20-kDa allergen was detected in dander extracts of several different dog breeds. However, the clinical relevance of this cross-sensitization needs to be confirmed. Nevertheless, the observation that more than 20% of cat- and dog-allergic patients mount IgE antibodies directed against a Fel d 1 cross-reactive dog allergen will have implications for diagnosis and therapy of IgE-mediated allergy to dogs.

Allergen-Specific Immunotherapy

Allergen-specific immunotherapy (ASIT), or allergy vaccination, is the only treatment able to change the natural course of allergic disease. Immunotherapy with cat dander extracts has proved efficacious for treatment of respiratory allergy to cat [62–64]. However, conventional ASIT performed with allergen extracts has several disadvantages. It is time consuming, linked to problems with compliance and there is a risk of treatment-associated adverse reactions. Therefore, great efforts have been placed into the search for new and safer regimens of ASIT. One approach is to base the therapy on single allergens prepared by recombinant DNA technology [65]. Moreover, single defined recombinant allergens may be designed to comprise properties that enhance the efficacy and minimize the risk of adverse side effects during treatment [65]. As discussed above, Fel d 1 is the dominant cause of allergic symptoms among patients sensitized to cat, and sensitization to Fel d 1 is a risk factor for developing asthma. Therefore, Fel d 1 is an excellent model allergen for the design of refined ASIT protocols.

Fel d 1 Hypoallergens

The most severe side effects of ASIT are caused by the binding of injected allergen to allergen-specific IgE on high-affinity FcεRI receptor-bearing effector cells, lead-

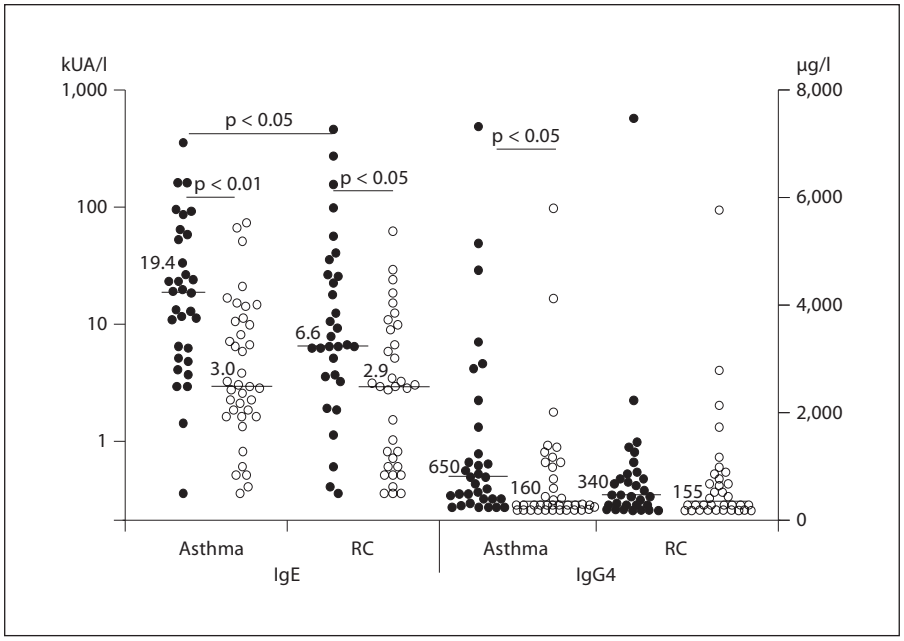

Fig. 3. Levels of IgE (kUA/l) and IgG4 (µg/l) antibodies to rFel d 1 (y-axis) in children (●) with asthma (n = 34) or rhinoconjunctivitis (RC; n = 33) to cat. Seventy-two cat-allergic adults (○) diagnosed with asthma (n = 38) or with RC (n = 34) were used for comparison. From Grönlund et al. [38], reprinted with permission from the publisher.

ing to cross-linking of the FcεRI receptors, degranulation and release of potent pro-inflammatory and anaphylactogenic mediators. In order to improve the safety of ASIT, the allergenicity of the allergen may be decreased by chemical or genetic modifications disrupting B-cell epitopes. T-cell epitopes should ideally be preserved so that the resulting hypoallergen will still be able to modify the allergen-specific immune response. The structure of Fel d 1 is known [33], and Fel d 1 has been extensively studied in terms of B- and T-cell epitopes [36, 42, 51] making it feasible to rationally design Fel d 1 hypoallergens. We have applied this approach on recombinant Fel d 1 [30] and created a series of Fel d 1 hypoallergen candidates with the aim to disrupt B-cell epitopes but leave T-cell epitopes intact (fig. 4). To achieve this, disulphide bridges were disrupted by site-directed mutagenesis of critical cysteine residues, and surface-exposed known T-cell epitopes were duplicated. Thus, by altering the 3-dimensional structure of Fel d 1, the capacity to bind IgE was reduced, while the T-cell-activating capacity remained unchanged or even enhanced. Altogether, 7 hypoallergen candidates were generated [66]. Three candidates, with 2 duplicated T-cell epitopes, and 1 or 2 disrupted disulphide bonds, displayed 400–900 times lower IgE-binding capacity in competition ELISA and induced a lower degree of basophil activation compared with Fel d 1. The hypoallergenic candidates were all able to stimulate T-cell proliferation of similar or larger magnitude than Fel d 1. Thus, the rationally designed Fel d 1 hypoallergens possess the desired properties of reduced IgE reactivity and retained T-cell-stimulating capacity and are promising candidates for use in ASIT to treat cat allergy. They will be further evaluated both in allergy models in mouse and in patient studies.

Peptide-Based Immunotherapy

An alternative way to directly target allergen-specific T cells without interaction with IgE is to construct T-cell epitope-containing allergen-derived peptides. In experimental models, synthetic peptides corresponding to immunodominant allergen T-cell epitopes have been shown to have the capability to induce antigen-specific tolerance [67, 68]. The concept was early applied on Fel d 1. Based on knowledge from T-cell epitope mapping studies of Fel d 1, 2 synthetic peptides of 27 amino acids comprising T-cell epitope-rich regions in Fel d 1 chain 1 were produced [36]. In the first clinical applications, the peptide vaccine, Allervax CAT, was shown to give a reduced symptom score after allergen challenge [69] and to shift the cytokine profile by decreasing the IL-4 production in Fel d 1-specific T cells [70, 71]. These effects were observed in patients receiving the highest peptide dose (750 µg weekly for 4–6 weeks), but high-dose therapy was also linked

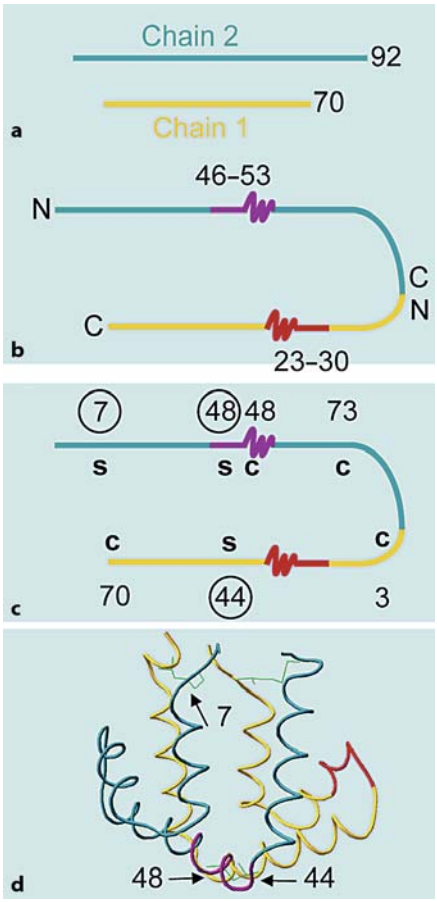

Fig. 4. Rational design of hypoallergens by the use of duplications of T-cell epitopes and point mutations. **a** Chains 1 and 2 of Fel d 1 contain 3 cysteine residues each. **b** The amino acids 46–53 on chain 2 and 23–30 on chain 1 were duplicated, and the 2 chains were joined to each other. **c** Cys44 on chain 1 and Cys7 and Cys48 on chain 2 were changed to serines. The construct with 3 cysteine mutations, rFel d 1, is exemplified. **d** A ribbon representation of the rFel d 1 backbone displays chain 1 in yellow and chain 2 in blue. The duplication of T-cell epitope modifications of chains 1 and 2 are shown in red and purple, respectively. The 3 disulphide bonds that link chains 1 and 2 are displayed in green, and cysteine residues subjected to mutagenesis are indicated by their respective numbers. From Saarne et al. [66], reprinted with permission from the publisher.

to adverse effects after repeated administration. Further investigations on patients treated with Allervax CAT failed to show clear clinical benefits and safety [72, 73]. The most common adverse reactions to the vaccine were late-phase events associated with respiratory symptoms, probably a result of activation of allergen-specific effector T cells, which may lead to late-phase asthmatic reactions independent of IgE [74].

A 'second generation' of Fel d 1 peptide vaccines was developed after the initial trials with Allervax CAT. The vaccine consisted of 12 shorter (16–17 residues) overlapping T-cell epitope-containing synthetic peptides capable of binding to a number of human leucocyte antigen molecules [75]. These peptides were used to treat 24 cat-allergic, asthmatic patients in a double-blind, placebo-controlled trial [76]. In this study, the primary outcome was measured as cutaneous reactions to whole cat dander extract. A reduced size of the late-phase skin reaction between baseline and follow-ups after 2 weeks and 3–9 months was detected. The treatment was also shown to reduce allergen-stimulated proliferation and Th1 and Th2 cytokine production, while IL-10 production was increased in vitro. In subsequent studies with the same 12 Fel d 1 peptides, an effect on the late-phase asthmatic response and on airway hyperresponsiveness was documented. In contrast to placebo-treated patients, the actively treated group displayed a reduced late asthmatic reaction to cat dander 3–4 months after treatment compared with baseline [77]. A treatment-associated reduction in airway hyperresponsiveness to histamine was also detected [78], indicating that peptide immunotherapy may be beneficial for treatment of cat-allergic asthma. These studies demonstrate that immunotherapy with Fel d 1-derived peptides are able to down-regulate allergen-specific Th2 responses and may be efficacious for treating cat allergen-induced asthma.

Engineered Fel d 1 Proteins with Immunomodulatory Function

Different strategies to achieve immunomodulation by engineering have been applied on Fel d 1. Common for these concepts of immunotherapy are that they involve linkage of the allergen to another molecule or carrier with an immunomodulatory function. Zhu and colleagues [79] have developed a strategy where they target the inhibitory Fcγ receptor FcγRIIb on mast cells and basophils. In contrast to FcεRI, which activates these cells, FcγRIIb has the ability to inhibit activation of the cell response (fig. 5). A fusion protein with preserved immunogenicity consisting of Fel d 1 and the Fc portion of IgG1 (Fcγ-Fel d 1) was produced with the hypothesis that the fusion protein should induce an allergen-specific inhibitory effect on mast cell and basophil degranulation. It was confirmed that Fcγ-Fel d 1 inhibited degranulation of human basophils in vitro and human FcεRI-transgenic mouse mast cells in vivo in an allergen-specific manner. The immu-

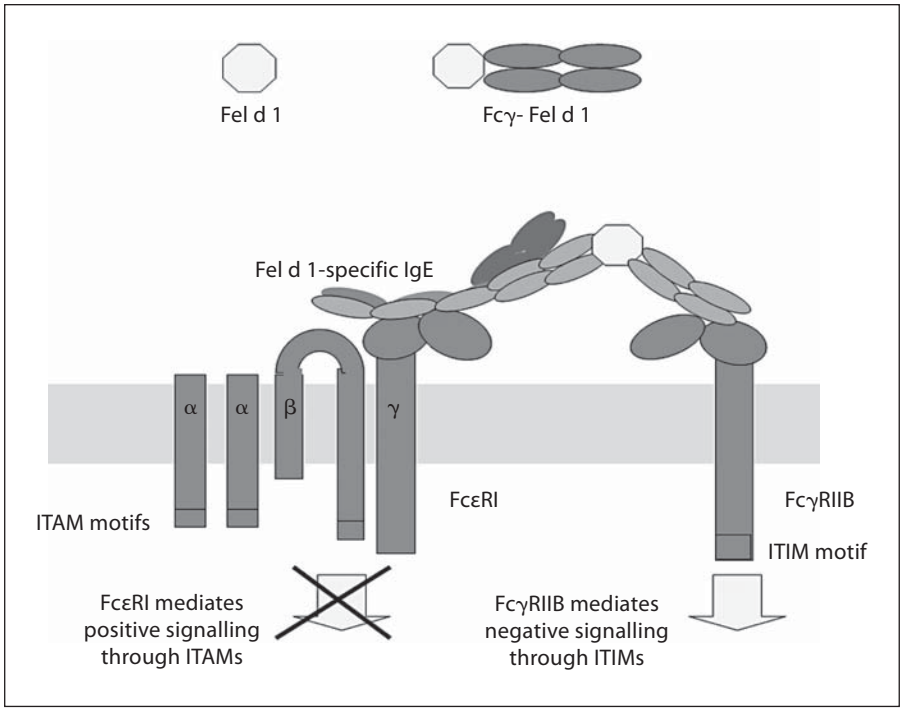

Fig. 5. Cross-linking of FcεRI receptors by free Fel d 1 bound to IgE normally leads to activation of the mast cells. The fusion protein Fcγ-Fel d 1 consists of the Fc portion of IgG1 linked to Fel d 1. When the fusion protein cross-links FcεRI and FcγRIIb, negative signalling through FcγRIIb inhibits mast cell activation in an antigen-specific manner.

notherapeutic potential of Fcγ-Fel d 1 was evaluated in mice sensitized to Fel d 1 [80]. Upon acute administration of Fcγ-Fel d 1 prior to Fel d 1 challenge, both local and systemic reactivity to Fel d 1 was blocked. In a protocol mimicking rush ASIT, sensitized mice were treated with 3 increasing subcutaneous doses of Fcγ-Fel d 1 on 3 consecutive days prior to intratracheal allergen challenge. This treatment led to inhibition of systemic (body temperature) and cutaneous reactivity to Fel d 1, measured 2 weeks after treatment. In addition, the rush ASIT protocol reduced allergen-induced hyperresponsiveness and inflammation in the airways. In contrast to administration of Fel d 1 alone, Fcγ-Fel d 1 did not induce any allergic response in the mice, probably as a result of negative signalling by FcγRIIb. Thus, Fcγ-Fel d 1 was shown to block Fel d 1-induced IgE-mediated reactivity in sensitized mice but did not trigger any allergic reactivity on its own. These results suggest that FcγRIIb activation in combination with ASIT is a promising therapeutic strategy [81].

A novel approach to target Fel d 1 to antigen-presenting cells (APCs) in immunotherapy was recently reported by Hulse et al. [82]. They linked recombinant Fel d 1 to a single chain fragment of a humanized anti-CD64 monoclonal antibody targeting the resulting fusion protein (termed 'H22-Fel d 1') to the high-affinity receptor for IgG (FcγRI) on APCs. The capacity of the fusion protein to modify the immune response to Fel d 1 was evaluated in an in vitro system with human monocyte-derived dendritic cells and CD4+ T cells isolated from cat-allergic or non-allergic subjects. Compared with Fel d 1 alone, H22-Fel d 1 induced increased numbers of IL-10+ and IL-5+ CD4+ cells. Modification of the T-cell response was only observed in cells from patients with allergy. The authors propose that targeting of allergen to FcγRI in immunotherapy may promote a regulated, protective, allergen-specific T-cell response.

We have developed a new adjuvant and allergen delivery system for ASIT and applied this approach on Fel d 1. It is based on covalent linkage of the allergen to agarose particles with a size of 2 μm in diameter. The rational for this approach is that the particles carrying large amounts of allergens should be phagocytosed by APCs, which will present the allergen-derived peptide-major histocompatibility complex II complexes at high density on the cell surface and exert immunomodulatory effects on allergen-specific T cells. In an initial study, it was shown that the particles, referred to as carbohydrate-based particles (CBP), exhibited beneficial properties for ASIT application when tested in vivo in naïve mice [83]. We then covalently coupled rFel d 1 to the particles (CBP-Fel d 1) and investigated the effect on human monocyte-derived dendritic cells [84]. We showed that CBP-Fel d 1 was readily ingested

by the dendritic cells in vitro and that a 'semi-mature' state of the cells was induced, possibly with the ability to induce regulated T-cell responses. To evaluate CBP-Fel d 1 for use in ASIT, we applied CBP-Fel d 1 in a mouse model for cat allergy. The mice received treatment with CBP-Fel d 1 (equivalent to 100 μg Fel d 1) in prophylactic and therapeutic treatment protocols [85, 86]. In the prophylactic protocol, the mice were treated with 3 subcutaneous injections of CBP-Fel d 1 prior to subcutaneous sensitization with recombinant Fel d 1 adsorbed to aluminium hydroxide and intranasal challenge with cat dander extract [85]. The treatment prevented induction of airway inflammation, induced allergen-specific T-cell anergy and rapid IgM and IgG1 responses. By tracking radiolabelled Fel d 1 after subcutaneous administration of CBP-Fel d 1, it was shown that the antigen remained up to 1 week at the injection site, in contrast to aluminium hydroxide-adsorbed Fel d 1, which was spread over the entire mouse already after 24 h [85]. Thus, prolonged exposure time might be one of the mechanisms by which CBP-Fel d 1 prevented allergic immune responses. CBP-Fel d 1 modified the allergic response also when applied in therapeutic treatment protocols, i.e. after sensitization and prior to allergen challenge [86]. The treated mice exhibited less airway inflammation and airway hyperresponsiveness, decreased levels of Fel d 1-specific IgE and increased levels of IgG compared with sham-treated mice. Taken together, linking Fel d 1 to CBP resulted in modulation of the allergen-specific immune response and the allergic response to Fel d 1 in vivo. Thus, covalent coupling of allergens to particles that are optimal for phagocytosis is a promising concept for application in improved protocols for ASIT.

Concluding Remarks

Fel d 1 is now available as an additional recombinant allergen and supplemental diagnostic tool for the diagnosis of cat allergy. This single major cat allergen, belonging to the uteroglobin protein family, has shown to be as good as cat dander extract in identifying patients. In the clinical setting, knowledge regarding the IgE levels in rFel d 1 may be useful as increased levels in children seem to be a potential risk factor for allergic asthma. Being the dominant cause of allergic symptoms to cat, Fel d 1 is an ideal model allergen for the development of new strategies for allergen-specific treatment of allergy and asthma. These include ASIT with hypoallergens, T-cell epitope-containing peptides and formulations consisting of Fel d 1 coupled to an immunomodulatory protein or carrier. Results reported from studies evaluating these approaches in experimental in vitro and in vivo systems as well as in clinical trials with Fel d 1 peptides bear promise that safer, more specific and efficient ways to treat cat-allergic patients will be available in the future.

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References

1 Ichikawa K, Iwasaki E, Baba M, Chapman MD: High prevalence of sensitization to cat allergen among Japanese children with asthma, living without cats. Clin Exp Allergy 1999;29:754–761.
2 Lau S, Illi S, Sommerfeld C, Niggemann B, Bergmann R, von Mutius E, Wahn U: Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Multicentre Allergy Study Group. Lancet 2000;356:1392–1397.
3 Freidhoff LR, Meyers DA, Marsh DG: A genetic-epidemiologic study of human immune responsiveness to allergens in an industrial population. 2. The associations among skin sensitivity, total serum IgE, age, sex, and the reporting of allergies in a stratified random sample. J Allergy Clin Immunol 1984;73:490–499.
4 Plaschke P, Janson C, Norrman E, Björnsson E, Lundbäck B, Lindholm N, Rosenhall L, Järvholm B, Boman G: Skin prick tests and specific IgE in adults from three different areas of Sweden. Allergy 1996;51:461–472.
5 Roost HP, Kunzli N, Schindler C, Jarvis D, Chinn S, Perruchoud AP, Ackermann-Liebrich U, Burney P, Wuthrich B: Role of current and childhood exposure to cat and atopic sensitization. European Community Respiratory Health Survey. J Allergy Clin Immunol 1999;104:941–947.
6 Almqvist C, Li Q, Britton WJ, Kemp AS, Xuan W, Tovey ER, Marks GB: Early predictors for developing allergic disease and asthma: examining separate steps in the 'allergic march'. Clin Exp Allergy 2007;37:1296–1302.
7 Schafer T, Ruhdorfer S, Weigl L, Wessner D, Heinrich J, Wichmann HE, Ring J: School education and allergic sensitization in adults. Allergy 2001;56:1206–1210.
8 Perzanowski MS, Rönmark E, Platts-Mills TA, Lundbäck B: Effect of cat and dog ownership on sensitization and development of asthma among preteenage children. Am J Respir Crit Care Med 2002;166:696–702.
9 Lau S, Illi S, Platts-Mills TA, Riposo D, Nickel R, Grüber C, Niggemann B, Wahn U, Multicentre Allergy Study Group: Longitudinal study on the relationship between cat allergen and endotoxin exposure, sensitization, cat-specific IgG and development of asthma in childhood – report of the German Multicentre Allergy Study (MAS 90). Allergy 2005;60:766–773.

10 Anderson MC, Baer H, Ohman JL Jr: A comparative study of the allergens of cat urine, serum, saliva, and pelt. J Allergy Clin Immunol 1985;76:563–569.
11 Løwenstein H, Lind P, Weeke B: Identification and clinical significance of allergenic molecules of cat origin. Part of the DAS 76 Study. Allergy 1985;40:430–441.
12 IUIS/WHO Allergen Nomenclature Sub-Committee. www.allergen.org.
13 Ohman JL Jr, Lowell FC, Bloch KJ: Allergens of mammalian origin. 3. Properties of a major feline allergen. J Immunol 1974;113: 1668–1677.
14 Ohman JL, Lowell FC, Bloch KJ: Allergens of mammalian origin: characterization of allergen extracted from cat pelts. J Allergy Clin Immunol 1973;52:231–241.
15 Spitzauer S, Pandjaitan B, Soregi G, Muhl S, Ebner C, Kraft D, Valenta R, Rumpold H: IgE cross-reactivities against albumins in patients allergic to animals. J Allergy Clin Immunol 1995;96:951–959.
16 Hilger C, Grigioni F, Hentges F: Sequence of the gene encoding cat *(Felis domesticus)* serum albumin. Gene 1996;169:295–296.
17 van Ree R, van Leeuwen WA, Bulder I, Bond J, Aalberse RC: Purified natural and recombinant Fel d 1 and cat albumin in in vitro diagnostics for cat allergy. J Allergy Clin Immunol 1999;104:1223–1230.
18 Ichikawa K, Vailes LD, Pomes A, Chapman MD: Molecular cloning, expression and modelling of cat allergen, cystatin (Fel d 3), a cysteine protease inhibitor. Clin Exp Allergy 2001;31:1279–1286.
19 Smith W, Butler AJ, Hazell LA, Chapman MD, Pomes A, Nickels DG, Thomas WR: Fel d 4, a cat lipocalin allergen. Clin Exp Allergy 2004;34:1732–1738.
20 Adedoyin J, Grönlund H, Oman H, Johansson SG, van Hage M: Cat IgA, representative of new carbohydrate cross-reactive allergens. J Allergy Clin Immunol 2007;119:640–645.
21 Adedoyin J, Johansson SG, Grönlund H, van Hage M: Interference in immunoassays by human IgM with specificity for the carbohydrate moiety of animal proteins. J Immunol Methods 2006;310:117–125.
22 Ohman JL Jr, Lowell FC: IgE antibody to cat allergens in an allergic population. J Allergy Clin Immunol 1977;60:317–323.
23 Kleine-Tebbe J, Kleine-Tebbe A, Jeep S, Schou C, Lowenstein H, Kunkel G: Role of the major allergen (Fel d I) in patients sensitized to cat allergens. Int Arch Allergy Immunol 1993;100:256–262.
24 Brown PR, Leitermann K, Ohman JL Jr: Distribution of cat allergen 1 in cat tissues and fluids. Int Arch Allergy Appl Immunol 1984; 74:67–70.
25 van Milligen FJ, Vroom TM, Aalberse RC: Presence of *Felis domesticus* allergen I in the cat's salivary and lacrimal glands. Int Arch Allergy Appl Immunol 1990;92:375–378.
26 de Groot H, van Swieten P, Aalberse RC: Evidence for a Fel d I-like molecule in the 'big cats' (Felidae species). J Allergy Clin Immunol 1990;86:107–116.
27 Charpin C, Mata P, Charpin D, Lavaut MN, Allasia C, Vervloet D: Fel d I allergen distribution in cat fur and skin. J Allergy Clin Immunol 1991;88:77–82.
28 Bartholome K, Kissler W, Baer H, Kopietz-Schulte E, Wahn U: Where does cat allergen 1 come from? J Allergy Clin Immunol 1985; 76:503–506.
29 Dabrowski AJ, Van der Brempt X, Soler M, Seguret N, Lucciani P, Charpin D, Vervloet D: Cat skin as an important source of Fel d I allergen. J Allergy Clin Immunol 1990;86: 462–465.
30 Grönlund H, Bergman T, Sandstrom K, Alvelius G, Reininger R, Verdino P, Hauswirth A, Liderot K, Valent P, Spitzauer S, Keller W, Valenta R, van Hage-Hamsten M: Formation of disulfide bonds and homodimers of the major cat allergen Fel d 1 equivalent to the natural allergen by expression in *Escherichia coli*. J Biol Chem 2003;278:40144–40151.
31 Duffort OA, Carreira J, Nitti G, Polo F, Lombardero M: Studies on the biochemical structure of the major cat allergen *Felis domesticus* I. Mol Immunol 1991;28:301–309.
32 Morgenstern JP, Griffith IJ, Brauer AW, Rogers BL, Bond JF, Chapman MD, Kuo MC: Amino acid sequence of Fel d I, the major allergen of the domestic cat: protein sequence analysis and cDNA cloning. Proc Natl Acad Sci USA 1991;88:9690–9694.
33 Kaiser L, Grönlund H, Sandalova T, Ljunggren HG, van Hage-Hamsten M, Achour A, Schneider G: The crystal structure of the major cat allergen Fel d 1, a member of the secretoglobin family. J Biol Chem 2003;278: 37730–37735.
34 Kaiser L, Velickovic TC, Badia-Martinez D, Adedoyin J, Thunberg S, Hallen D, Berndt K, Grönlund H, Gafvelin G, van Hage M, Achour A: Structural characterization of the tetrameric form of the major cat allergen Fel d 1. J Mol Biol 2007;370:714–727.
35 Kaiser L, Grönlund H, Sandalova T, Ljunggren HG, Achour A, Schneider G, van Hage-Hamsten M: Three-dimensional structure of Fel d 1, the major allergen in cat. Int Arch Allergy Immunol 2003;132:25–26.
36 Keating KM, Segal DB, Craig SJ, Nault AK, Semensi V, Wasserman AS, Counsell CM, Bond JF: Enhanced immunoreactivity and preferential heterodimer formation of reassociated Fel d I recombinant chains. Mol Immunol 1995;32:287–293.
37 van Neerven RJ, van de Pol MM, van Milligen FJ, Jansen HM, Aalberse RC, Kapsenberg ML: Characterization of cat dander-specific T lymphocytes from atopic patients. J Immunol 1994;152:4203–4210.
38 Grönlund H, Adedoyin J, Reininger R, Varga EM, Zach M, Fredriksson M, Kronqvist M, Szepfalusi Z, Spitzauer S, Gronneberg R, Valenta R, Hedlin G, van Hage M: Higher immunoglobulin E antibody levels to recombinant Fel d 1 in cat-allergic children with asthma compared with rhinoconjunctivitis. Clin Exp Allergy 2008;38:1275–1281.
39 de Groot H, van Swieten P, van Leeuwen J, Lind P, Aalberse RC: Monoclonal antibodies to the major feline allergen Fel d I. 1. Serologic and biologic activity of affinity-purified Fel d I and of Fel d I-depleted extract. J Allergy Clin Immunol 1988;82:778–786.
40 Chapman MD, Aalberse RC, Brown MJ, Platts-Mills TA: Monoclonal antibodies to the major feline allergen Fel d I. 2. Single step affinity purification of Fel d I, N-terminal sequence analysis, and development of a sensitive two-site immunoassay to assess Fel d I exposure. J Immunol 1988;140:812–818.
41 Batard T, Bukovec F, Berrouet C, Destombes V, Didierlaurent A, Andre C: Demonstration of a partially cryptic epitope of the major cat allergen Fel d 1: consequences for mAb-based standardization of cat extracts. J Allergy Clin Immunol 2000;106:669–676.
42 Kristensen AK, Schou C, Roepstorff P: Determination of isoforms, N-linked glycan structure and disulfide bond linkages of the major cat allergen Fel d 1 by a mass spectrometric approach. Biol Chem 1997;378:899–908.
43 Beato M: Binding of steroids to uteroglobin. J Steroid Biochem 1976;7:327–334.
44 Griffith IJ, Craig S, Pollock J, Yu XB, Morgenstern JP, Rogers BL: Expression and genomic structure of the genes encoding FdI, the major allergen from the domestic cat. Gene 1992;113:263–268.
45 Klug J, Beier HM, Bernard A, Chilton BS, Fleming TP, Lehrer RI, Miele L, Pattabiraman N, Singh G: Uteroglobin/Clara cell 10-kDa family of proteins: nomenclature committee report. Ann NY Acad Sci 2000;923:348–354.
46 Gillner M, Lund J, Cambillau C, Alexandersson M, Hurtig U, Bergman A, Klasson-Wehler E, Gustafsson JA: The binding of methylsulfonyl-polychloro-biphenyls to uteroglobin. J Steroid Biochem 1988;31:27–33.
47 Hard T, Barnes HJ, Larsson C, Gustafsson JA, Lund J: Solution structure of a mammalian PCB-binding protein in complex with a PCB. Nat Struct Biol 1995;2:983–989.
48 Emes RD, Riley MC, Laukaitis CM, Goodstadt L, Karn RC, Ponting CP: Comparative evolutionary genomics of androgen-binding protein genes. Genome Res 2004;14:1516–1529.
49 van Milligen FJ, van 't Hof W, van den Berg M, Aalberse RC: IgE epitopes on the cat *(Felis domesticus)* major allergen Fel d I: a study with overlapping synthetic peptides. J Allergy Clin Immunol 1994;93:34–43.
50 van 't Hof W, van Milligen FJ, van den Berg M, Lombardero M, Chapman MD, Aalberse RC: Epitope mapping of the cat *(Felis domesticus)* major allergen Fel d I by overlapping synthetic peptides and monoclonal antibodies against native and denatured Fel d I. Allergy 1993;48:255–263.
51 Vailes LD, Li Y, Bao Y, DeGroot H, Aalberse RC, Chapman MD: Fine specificity of B-cell epitopes on *Felis domesticus* allergen I (Fel d I): effect of reduction and alkylation or deglycosylation on Fel d I structure and antibody binding. J Allergy Clin Immunol 1994; 93:22–33.

52 van Milligen FJ, van 't Hof W, Aalberse RC: IgE and IgG4 binding to synthetic peptides of the cat *(Felis domesticus)* major allergen Fel d I. Int Arch Allergy Immunol 1994;103: 274–279.
53 Bond JF, Brauer AW, Segal DB, Nault AK, Rogers BL, Kuo MC: Native and recombinant Fel d I as probes into the relationship of allergen structure to human IgE immunoreactivity. Mol Immunol 1993;30:1529–1541.
54 Counsell CM, Bond JF, Ohman JL Jr, Greenstein JL, Garman RD: Definition of the human T-cell epitopes of Fel d 1, the major allergen of the domestic cat. J Allergy Clin Immunol 1996;98:884–894.
55 Mark PG, Segal DB, Dallaire ML, Garman RD: Human T and B cell immune responses to Fel d 1 in cat-allergic and non-cat-allergic subjects. Clin Exp Allergy 1996;26:1316–1328.
56 Bateman EAL, Ardern-Jones MR, Ogg GS: Identification of an immunodominant region of Fel d 1 and characterization of constituent epitopes. Clin Exp Allergy 2008;38: 1760–1768.
57 Haselden BM, Syrigou E, Jones M, Huston D, Ichikawa K, Chapman MD, Kay AB, Larche M: Proliferation and release of IL-5 and IFN-gamma by peripheral blood mononuclear cells from cat-allergic asthmatics and rhinitics, non-cat-allergic asthmatics, and normal controls to peptides derived from Fel d 1 chain 1. J Allergy Clin Immunol 2001;108: 349–356.
58 Reefer AJ, Carneiro RM, Custis NJ, Platts-Mills TA, Sung SS, Hammer J, Woodfolk JA: A role for IL-10-mediated HLA-DR7-restricted T cell-dependent events in development of the modified Th2 response to cat allergen. J Immunol 2004;172:2763–2772.
59 Pandjaitan B, Swoboda I, Brandejsky-Pichler F, Rumpold H, Valenta R, Spitzauer S: *Escherichia coli* expression and purification of recombinant dog albumin, a cross-reactive animal allergen. J Allergy Clin Immunol 2000; 105:279–285.
60 Spitzauer S, Schweiger C, Sperr WR, Pandjaitan B, Valent P, Muhl S, Ebner C, Scheiner O, Kraft D, Rumpold H, et al: Molecular characterization of dog albumin as a cross-reactive allergen. J Allergy Clin Immunol 1994;93:614–627.
61 Reininger R, Varga EM, Zach M, Balic N, Lindemeier AD, Swoboda I, Grönlund H, van Hage M, Rumpold H, Valenta R, Spitzauer S: Detection of an allergen in dog dander that cross-reacts with the major cat allergen, Fel d 1. Clin Exp Allergy 2007;37: 116–124.
62 Varney VA, Edwards J, Tabbah K, Brewster H, Mavroleon G, Frew AJ: Clinical efficacy of specific immunotherapy to cat dander: a double-blind placebo-controlled trial. Clin Exp Allergy 1997;27:860–867.
63 Ewbank PA, Murray J, Sanders K, Curran-Everett D, Dreskin S, Nelson HS: A double-blind, placebo-controlled immunotherapy dose-response study with standardized cat extract. J Allergy Clin Immunol 2003;111: 155–161.
64 Nanda A, O'Connor M, Anand M, Dreskin SC, Zhang L, Hines B, Lane D, Wheat W, Routes JM, Sawyer R, Rosenwasser LJ, Nelson HS: Dose dependence and time course of the immunologic response to administration of standardized cat allergen extract. J Allergy Clin Immunol 2004;114:1339–1344.
65 Niederberger V, Valenta R: Molecular approaches for new vaccines against allergy. Expert Rev Vaccines 2006;5:103–110.
66 Saarne T, Kaiser L, Grönlund H, Rasool O, Gafvelin G, van Hage-Hamsten M: Rational design of hypoallergens applied to the major cat allergen Fel d 1. Clin Exp Allergy 2005; 35:657–663.
67 Ali FR, Larche M: Peptide-based immunotherapy: a novel strategy for allergic disease. Expert Rev Vaccines 2005;4:881–889.
68 Larche M: Peptide therapy for allergic diseases: basic mechanisms and new clinical approaches. Pharmacol Ther 2005;108:353–361.
69 Van Metre TE Jr, Marsh DG, Adkinson NF Jr, Fish JE, Kagey-Sobotka A, Norman PS, Radden EB Jr, Rosenberg GL: Dose of cat *(Felis domesticus)* allergen 1 (Fel d 1) that induces asthma. J Allergy Clin Immunol 1986; 78:62–75.
70 Marcotte GV, Braun CM, Norman PS, Nicodemus CF, Kagey-Sobotka A, Lichtenstein LM, Essayan DM: Effects of peptide therapy on ex vivo T-cell responses. J Allergy Clin Immunol 1998;101:506–513.
71 Pene J, Desroches A, Paradis L, Lebel B, Farce M, Nicodemus CF, Yssel H, Bousquet J: Immunotherapy with Fel d 1 peptides decreases IL-4 release by peripheral blood T cells of patients allergic to cats. J Allergy Clin Immunol 1998;102:571–578.
72 Chan-Yeung M, Manfreda J, Dimich-Ward H, Lam J, Ferguson A, Warren P, Simons E, Broder I, Chapman M, Platts-Mills T, et al: Mite and cat allergen levels in homes and severity of asthma. Am J Respir Crit Care Med 1995;152:1805–1811.
73 Burr ML, Limb ES, Maguire MJ, Amarah L, Eldridge BA, Layzell JC, Merrett TG: Infant feeding, wheezing, and allergy: a prospective study. Arch Dis Child 1993;68:724–728.
74 Haselden BM, Kay AB, Larche M: Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. J Exp Med 1999;189:1885–1894.
75 Oldfield WL, Kay AB, Larche M: Allergen-derived T cell peptide-induced late asthmatic reactions precede the induction of antigen-specific hyporesponsiveness in atopic allergic asthmatic subjects. J Immunol 2001; 167:1734–1739.
76 Oldfield WL, Larche M, Kay AB: Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. Lancet 2002;360:47–53.
77 Alexander C, Tarzi M, Larche M, Kay AB: The effect of Fel d 1-derived T-cell peptides on upper and lower airway outcome measurements in cat-allergic subjects. Allergy 2005;60:1269–1274.
78 Alexander C, Ying S, Kay AB, Larche M: Fel d 1-derived T cell peptide therapy induces recruitment of CD4+ CD25+; CD4+ interferon-gamma+ T helper type 1 cells to sites of allergen-induced late-phase skin reactions in cat-allergic subjects. Clin Exp Allergy 2005;35:52–58.
79 Zhu D, Kepley CL, Zhang K, Terada T, Yamada T, Saxon A: A chimeric human-cat fusion protein blocks cat-induced allergy. Nat Med 2005;11:446–449.
80 Terada T, Zhang K, Belperio J, Londhe V, Saxon A: A chimeric human-cat Fcgamma-Fel d 1 fusion protein inhibits systemic, pulmonary, and cutaneous allergic reactivity to intratracheal challenge in mice sensitized to Fel d 1, the major cat allergen. Clin Immunol 2006;120:45–56.
81 Saxon A, Kepley C, Zhang K: 'Accentuate the negative, eliminate the positive': engineering allergy therapeutics to block allergic reactivity through negative signaling. J Allergy Clin Immunol 2008;121:320–325.
82 Hulse KE, Reefer AJ, Engelhard VH, Satinover SM, Patrie JT, Chapman MD, Woodfolk JA: Targeting Fel d 1 to FcgammaRI induces a novel variation of the T(H)2 response in subjects with cat allergy. J Allergy Clin Immunol 2008;121:756–762.e4.
83 Grönlund H, Vrtala S, Wiedermann U, Dekan G, Kraft D, Valenta R, Van Hage-Hamsten M: Carbohydrate-based particles: a new adjuvant for allergen-specific immunotherapy. Immunology 2002;107:523–529.
84 Andersson TN, Ekman GJ, Grönlund H, Buentke E, Eriksson TL, Scheynius A, Van Hage-Hamsten M, Gafvelin G: A novel adjuvant-allergen complex, CBP-rFel d 1, induces up-regulation of CD86 expression and enhances cytokine release by human dendritic cells in vitro. Immunology 2004;113:253–259.
85 Thunberg S, Neimert-Andersson T, Cheng Q, Wermeling F, Bergstrom U, Swedin L, Dahlen SE, Arner E, Scheynius A, Karlsson MC, Gafvelin G, van Hage M, Grönlund H: Prolonged antigen-exposure with carbohydrate particle based vaccination prevents allergic immune responses in sensitized mice. Allergy 2009;64:919–926.
86 Neimert-Andersson T, Thunberg S, Swedin L, Wiedermann U, Jacobsson-Ekman G, Dahlen SE, Scheynius A, Grönlund H, van Hage M, Gafvelin G: Carbohydrate-based particles reduce allergic inflammation in a mouse model for cat allergy. Allergy 2008;63: 518–526.