

Mechanisms of allergen-specific desensitization

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Background: Allergen-specific desensitization (SIT) is the most effective therapy for allergies. Although allergen-specific antibodies have an important role in the process, mechanisms of IgG-mediated inhibition of allergic reactions are not well defined.

Objective: We investigated mechanisms by which SIT-induced allergen-specific IgGs inhibit allergic reactions.

Methods: We generated mAbs that recognize 3 nonoverlapping epitopes of the major cat allergen Fel d 1. Each of the mAbs was produced as an IgE and different IgG isotype.

Results: IgEs against 2 nonoverlapping epitopes on Fel d 1 are necessary and sufficient to sensitize mast cells for maximal FcεRI signaling and degranulation on exposure to monomeric Fel d 1. IgE antibodies of a third specificity did not further increase mast cell degranulation, indicating that formation of large FcεRI clusters are not required to induce maximal activation of mast cells. A single IgG that was specific for an epitope different from those recognized by the IgEs was a potent inhibitor of Fel d 1–mediated mast cell activation *in vitro* and *in vivo*. This inhibition required Fcγ receptor-IIB. In human beings, IgGs of a single specificity were able to block degranulation of basophils from individuals with cat allergy. The inhibitory potential of these antibodies increased when larger allergen-IgG complexes were formed.

Conclusions: These data reconcile conflicting theories in the literature and might explain the reason IgE levels do not necessarily decrease during therapy, despite clinical efficacy. These findings have important implications for vaccine design. (J Allergy Clin Immunol 2010;■■■:■■■-■■■.)

Key words: Allergy, vaccination, FcγRIIB-mediated inhibition, mast cell activation requirements, Fel d 1, mAbs

The prevalence of IgE-mediated allergies, such as rhinoconjunctivitis and asthma, has increased dramatically in industrialized countries over the last century; as much as 30% of the population is currently allergic to pollen, house dust mites, or

Abbreviations used

BMMC: Bone marrow–derived mast cell
FACS: Fluorescence-activated cell sorting
Fcγ RIIB: Fcγ receptor-IIB
IC: Immune complexes
rFel d 1: Recombinant Fel d 1
SIT: Allergen-specific immunotherapy
VLP: Virus-like particle

animal dander.^{1,2} Drugs that are used to treat allergies, such as antihistamines or corticosteroids, ameliorate symptoms but do not stop progression; the only therapy that modifies progression of allergies is allergen-specific immunotherapy (SIT). SIT has major disadvantages, however, in that it requires numerous allergen administrations over 3 to 5 years^{3,4} and can cause severe adverse events that range from local allergic reactions to anaphylaxis.⁵ SIT has been reported to modify different aspects of the immune system, inducing a shift of allergy-promoting T_H2 cells to T_H1 cells,⁶ epitope-specific T-cell anergy,⁷ and allergen-specific regulatory T cells that suppress the responses of effector T cells.⁸ Furthermore, SIT has been reported to induce allergen-specific IgGs, mainly of the IgG₁ and IgG₄ subclasses.^{9,10}

Mast cells constitutively express FcεRI, which mediates allergic reactions. Their activation, via FcεRI, induces type I hypersensitivity reactions that range from mild rhinoconjunctivitis to acute asthmatic responses. Cross-linking of receptor-bound IgEs by allergen signals mast cells to release vasoactive amines and cytokines¹¹ (a process termed *degranulation*) and synthesize proinflammatory lipids,¹² cytokines, and chemokines.¹³

It is assumed that IgGs induced by allergen-specific immunotherapy can inhibit IgE-mediated mast cell degranulation—the so-called “blocking antibodies” compete with IgE for allergen binding.¹⁴ However, there is also evidence that IgGs prevent allergic reactions by a different mechanism. IgE-induced degranulation has been shown to be inhibited on coaggregation of FcεRI and Fcγ receptor IIB (FcγRIIB) *in vitro*.¹⁵ FcγRIIB-deficient mice are more susceptible to allergic rhinitis¹⁶ and have increased anaphylactic responses compared with controls.¹⁷ Furthermore, fusion of the major cat allergen Fel d 1 to human Fcγ1 has been reported to reduce allergic reactions in mice with allergies to Fel d 1.¹⁸ Taken together, these findings indicate a role for allergen-specific IgGs beyond neutralization. However, the concept of FcγRIIB-dependent negative regulation remains controversial. For instance, FcγRIIB does not prevent mast cell activation in desensitized, allergic mice.¹⁹ Furthermore, IgG induced by SIT blocks mast cell activation in an FcγRIIB-independent manner.²⁰

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METHODS

Mice

Balb/c mice were purchased from Harlan (Horst, The Netherlands) at the age of 6 weeks. Balb/c Fc γ RIIB^{-/-} mice (N12) were purchased from Taconic Farms, Inc, Denmark, at the age of 5 to 7 weeks.

Cloning, expression, purification, and coupling of recombinant Fel d 1

A modified covalent fusion of recombinant Fel d 1 (rFel d 1) was generated as previously described.^{19,21} Briefly, a cDNA that encoded a covalent dimer of chains 2 and 1 of Fel d 1, spaced by a 15 aa-linker (GGGGS)₃, was obtained by PCR. This amplicon was cloned into a modified version of pET-42a(+) leading to the addition of the sequence LEHHHHHHGGC at the C-terminus of the covalent dimer. This sequence contains a His-tag followed by a GGC linker used for coupling of the protein to Q β (pET-42T $\times$ Fel d 1-15aa-HC). The *Escherichia coli* strain BL21(DE3) was transformed with pET-42T $\times$ Fel d 1-15aa-HC. rFel d 1 was purified by Ni²⁺ affinity chromatography, and native folding was achieved by intramolecular reshuffling of the disulfide bonds with oxidized glutathione (Applichem) and reduced glutathione (Applichem) at a molar ratio of 1:1. Refolded rFel d 1 was further purified by size-exclusion chromatography (Superdex 75 pg; GE Healthcare). Virus-like particles (VLPs) derived from the bacteriophage Q β were expressed in an *E coli* strain (JM109) that carried the expression plasmid pQ10 and purified as previously described.²² The coupling of rFel d 1 to the Q β -VLP was described previously in detail.¹⁹

Cloning, expression, and purification of mouse mAbs

Balb/c mice were immunized with 50 μ g Q β -rFel d 1. Fel d 1-specific memory B cells were isolated by fluorescence-activated cell sorting (FACS). The sorted B cells were used to generate a random combinatorial antibody library, which was screened by Sindbis-based mammalian cell display as described previously.²³ Three antibodies that recognized different epitopes on Fel d 1 were identified and named A044, F127, and G078. Each of the antibodies was produced as mouse isotypes IgG₁, IgG_{2a}, and IgE, and as chimeric mouse/human antibodies of the IgG₁ and IgG₄ isotypes. Antibodies were expressed in 293T cells and purified from culture supernatants by affinity chromatography over a protein A-Sepharose column (GE Healthcare).

Fel d 1-ELISA

ELISA plates were coated with 1 μ g/mL rFel d 1. Purified antibodies from the clones A044, F127, and G078 (expressed as IgG₁, IgG_{2a}, or IgE) were added to the first well at 1 μ g/mL and titrated as indicated in the descriptions of experiments. The antibody binding was detected by horseradish peroxidase-conjugated antimouse IgG₁ (BD), antimouse IgG_{2a} (Southern Bioscience), or antimouse IgE (Southern Biotech), respectively.

Competition ELISA

ELISA plates were coated with 1 μ g/mL IgE A044, and then monomeric rFel d 1 (1 μ g/mL) was added for 1 hour. IgG_{2a} F127 was then added at a concentration of 10 μ g/mL, and the plates were incubated for 30 minutes before the addition of 1 μ g/mL IgG₁ G078. As controls, either IgG_{2a} F127 (10 μ g/mL) or IgG₁ G078 (1 μ g/mL) was added to the IgE A044-rFel d 1 complex on the plates. Antibody binding was detected by horseradish peroxidase-conjugated antimouse IgG₁ (BD) or antimouse IgG_{2a} (Southern Bioscience).

Bone marrow-derived mast cells

Bone marrow-derived mast cells (BMMCs) were generated from bone marrow cells that had been cultured at 5×10^6 cells in complete RPMI medium supplemented with recombinant mouse IL-3 (30 ng/mL; R&D Systems) and recombinant mouse stem cell factor (50 ng/mL; R&D Systems). Four weeks later, the differentiation of BMMCs was monitored by measuring the

expression of c-Kit (allophycocyanin-antimouse c-Kit; BD) and Fc ϵ RI (phycoerythrin-antimouse Fc ϵ RI; eBioscience) by flow cytometry.

BMMC degranulation

Bone marrow-derived mast cells were incubated with Fel d 1-specific mouse IgE for 1 hour at 4°C (2 μ g/mL clone A044, F127, and/or G078). The cells were washed once with Tyrods buffer (0.1% BSA, 0.1% glucose, 10 mmol/L HEPES, 130 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, pH 7.4) and incubated with rFel d 1 alone or rFel d 1-IgG complexes at the indicated concentrations for 1 hour at 37°C. The degranulation was stopped by placing the plate on ice. After spinning down BMMCs, supernatants were collected. The cells were lysed with Tyrods buffer that contained 0.5% Triton X-100 (Sigma). The degree of degranulation was determined by measuring the release of β -hexosaminidase. Supernatants and cell pellets were incubated with the substrate p-nitrophenyl-N-acetyl-p-D-glucosamine (Sigma) in 0.1 mol/L sodium citrate buffer (pH 4.5) for 90 minutes at 37°C. The reaction was terminated by the addition of 0.2 mol/L glycine (pH 10.7) and the OD at 405 nm determined. The degree of degranulation was calculated as follows:

$$\% \text{ of degranulation} = \text{OD}_{\text{supernatant}} / (\text{OD}_{\text{supernatant}} + \text{OD}_{\text{lysed cells}}).$$

Calcium flux

Bone marrow-derived mast cells 5×10^6 /mL were stained with 4 μ g/mL Fluo-4 (BD) and 10 μ g/mL FuraRed (BD) for 45 minutes at 37°C. The cells were washed twice with Tyrods buffer. The stained cells were incubated with Fel d 1-specific mouse IgE for 30 minutes at 4°C (2 μ g/mL clone A044, F127, and/or G078) and washed twice with Tyrods buffer. BMMCs 2×10^6 were resuspended in 200 μ L Tyrods buffer (37°C). Calcium flux was induced by adding 100 ng/mL rFel d 1 and was measured by flow cytometry.

Flow cytometry

Bone marrow-derived mast cells were washed with FACS buffer (2% FCS in PBS) and incubated with rFel d 1-IgG₁ or rFel d 1-IgG_{2a} complexes, which consisted of different combinations of IgGs, for 30 minutes at 4°C. Afterward, BMMCs were washed twice with FACS buffer and stained with either phycoerythrin-antimouse-IgG₁ (clone A85-1; BD) or fluorescein isothiocyanate-antimouse-IgG_{2a} (clone R19-15; BD) antibodies for 20 minutes at 4°C. Cells were then washed twice with FACS buffer and measured by flow cytometry.

Ear prick test

Mice were given intravenous injections of 10 μ g mouse IgE (clone A044, F127, and/or G078) and/or 100 μ g IgG (clone A044, F127, and/or G078). Twenty-four hours later, each mouse was given an intravenous injection of 200 μ L Evans blue dye (0.5% in PBS); 20 minutes later, a drop of rFel d 1 (15 μ g/20 μ L in PBS) was placed on the outer ear skin of the anesthetized mice, and their ears were pricked with a needle. The degranulation of mast cells was determined 45 minutes after the prick and quantified by the degree of dye extravasation. Briefly, mice were killed and the ears collected. The dye was extracted from the collected ears by digestion with 150 μ L 1 mol/L KOH overnight at 37°C. The next day, 150 μ L 5% H₃PO₄ in acetone was added, and the samples were centrifuged. Supernatants were collected and measured at an OD of 595 nm to quantify the extracted dye.

Human basophil degranulation assay

Heparinized blood (100 μ L) from donors who were allergic to cats was incubated with 20 μ L basophil stimulation buffer,²⁴ with the indicated concentration of rFel d 1 or rFel d 1-human IgG complexes, for 20 minutes at 37°C. The degranulation was stopped by chilling the cells on ice. Cells were stained with fluorescein isothiocyanate-anti-CD63, phycoerythrin-anti-CD123, and peridinin chlorophyll protein-anti-HLA-DR antibodies (BD) for 20 minutes before erythrocytes were lysed with FACS lysis buffer (BD).

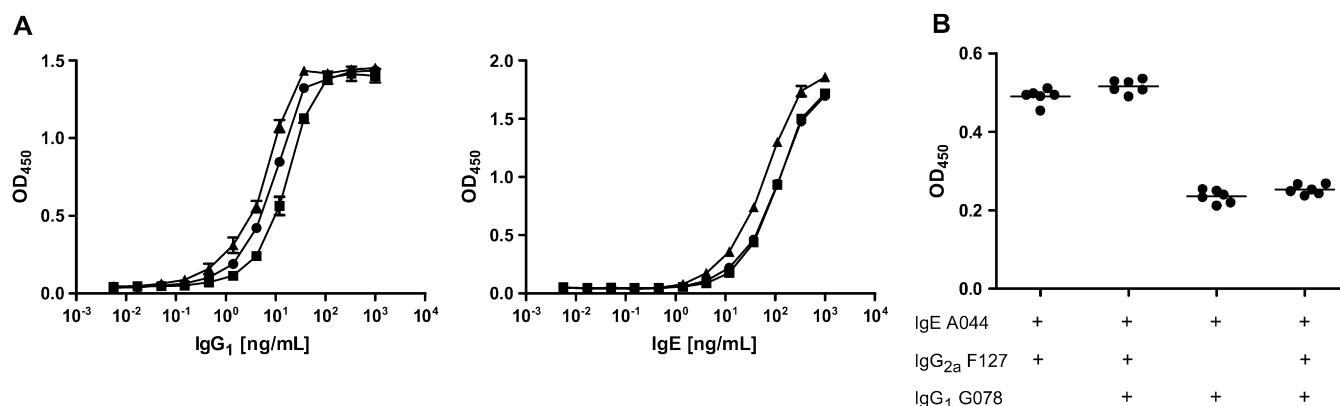


FIG 1. mAbs display similar binding properties to rFel d 1 and recognize distinct epitopes. **A**, Binding properties of the 3 mAb clones (A044 ♦, F127 ■, and G078 ▲) to monomeric rFel d 1, assessed by ELISA. The mAbs displayed similar properties of binding to monomeric rFel d 1, regardless of isotype. **B**, Sequential addition of the different clones and isotypes, as indicated in Methods, yielded results similar to those of any of the mAbs alone in a competition ELISA. These findings indicate that the clones recognize distinct epitopes on monomeric rFel d 1. Data represent 3 or more independent experiments.

Fixed blood cells were washed twice with PBS and analyzed by flow cytometry.

RESULTS

Binding properties of Fel d 1-specific antibodies

To investigate the mechanism by which allergen-specific IgGs block type I allergic reactions and influence IgE-induced degranulation of mast cells, we generated a panel of mAbs specific for the major cat allergen Fel d 1. Briefly, mice were immunized with monomeric rFel d 1 displayed on bacteriophage Q β -derived VLPs.¹⁹ Several antibodies were isolated from these mice by mammalian cell display as described previously.²³ mAbs A044, F127, and G078, which are specific for rFel d 1, were identified and expressed as mouse IgE, IgG₁, and IgG_{2a}. These mAbs bound rFel d 1 similarly in an ELISA (Fig 1, A, and not shown) and displayed affinities that ranged from 0.3 to 0.6 nmol/L for rFel d 1 (determined by Friguet ELISA; data not shown).²⁵ To identify the epitopes that these antibodies recognize, competition ELISAs were performed with monomeric Fel d 1 by using IgE-A044 as a capture antibody; IgG_{2a} F127 and IgG₁ G078 were then added, alone or together. Bound antibodies were detected with either horseradish peroxidase-conjugated anti-IgG_{2a} or anti-IgG₁. As shown in Fig 1, B, the 3 antibodies were able to bind monomeric rFel d 1 simultaneously, so they recognize different nonoverlapping epitopes. These antibodies, which bind Fel d 1 with similar affinities, represent ideal tools for the study of IgE-induced mast cell degranulation.

Antibodies that recognize 2 epitopes suffice for BMMC degranulation

Mast cells release prestored inflammatory mediators on cross-linking of their Fc ϵ Rs by allergen-bound IgE.¹¹ However, the requirements for degranulation have not been studied in detail. By using the panel of Fel d 1-specific mAbs, we investigated the size and type of allergen-IgE complexes required to induce mast cell degranulation. BMMCs were loaded either with 1, 2, or 3 different monoclonal IgEs and then challenged with monomeric rFel d 1. When cells were loaded with any of the mAbs alone, monomeric rFel d 1 induced neither degranulation nor

calcium flux (Fig 2, A and C). In contrast, monomeric rFel d 1 induced strong BMMC degranulation and calcium flux in cells that had been loaded with 2 different IgEs. This activity did not increase further after addition of an IgE of a third specificity (Fig 2, A and C). Two-dimensional strings of IgE-allergen complexes are therefore sufficient to induce the maximum amount of degranulation of BMMCs. Formation of large clusters by 3 IgE antibodies that recognized different nonoverlapping epitopes did not further increase the response. In agreement with these findings, dimeric rFel d 1 induced BMMC degranulation, which had been loaded with a single IgE (Fig 2, B). To explore these findings further *in vivo*, Balb/c mice were given intravenous injections of either 1 or 2 monoclonal IgEs. In agreement with the *in vitro* data, the skin prick test with monomeric rFel d 1 induced strong dye extravasation only in mice that had been injected with at least 2 different monoclonal IgEs. Likewise, addition of an IgE that recognized a third, nonoverlapping epitope did not increase the degree of degranulation induced by the allergen challenge (Fig 2, D). Therefore, 2-dimensional strings of IgE-allergen complexes are sufficient to induce the maximum amount of mast cell degranulation.

IgG–Fel d 1 complexes inhibit IgE-mediated BMMC degranulation

Mast cells express Fc γ RIIB, an inhibitory receptor that binds to IgG-antigen immune complexes (ICs). Having established the requirements for IgEs to induce mast cell degranulation, we investigated the role of allergen-specific IgGs. As expected, monomeric rFel d 1 induced degranulation of BMMCs that were loaded with 2 different IgE mAbs (A044 and F127). rFel d 1–induced degranulation was efficiently inhibited by either of the neutralizing IgGs (A044 or F127) that had been used to load BMMCs (data not shown). BMMCs were also loaded with IgEs of 2 different specificities (A044 or F127) and exposed to IC made of monomeric rFel d 1 with IgGs (IgG₁ or IgG_{2a}) of the third specificity (G078). Preincubation of monomeric rFel d 1 with IgG G078 resulted in a dose-dependent reduction in rFel d 1–induced BMMC degranulation, independent of the IgG subclass (Fig 3, A). Similar results were obtained for other combinations (not shown).

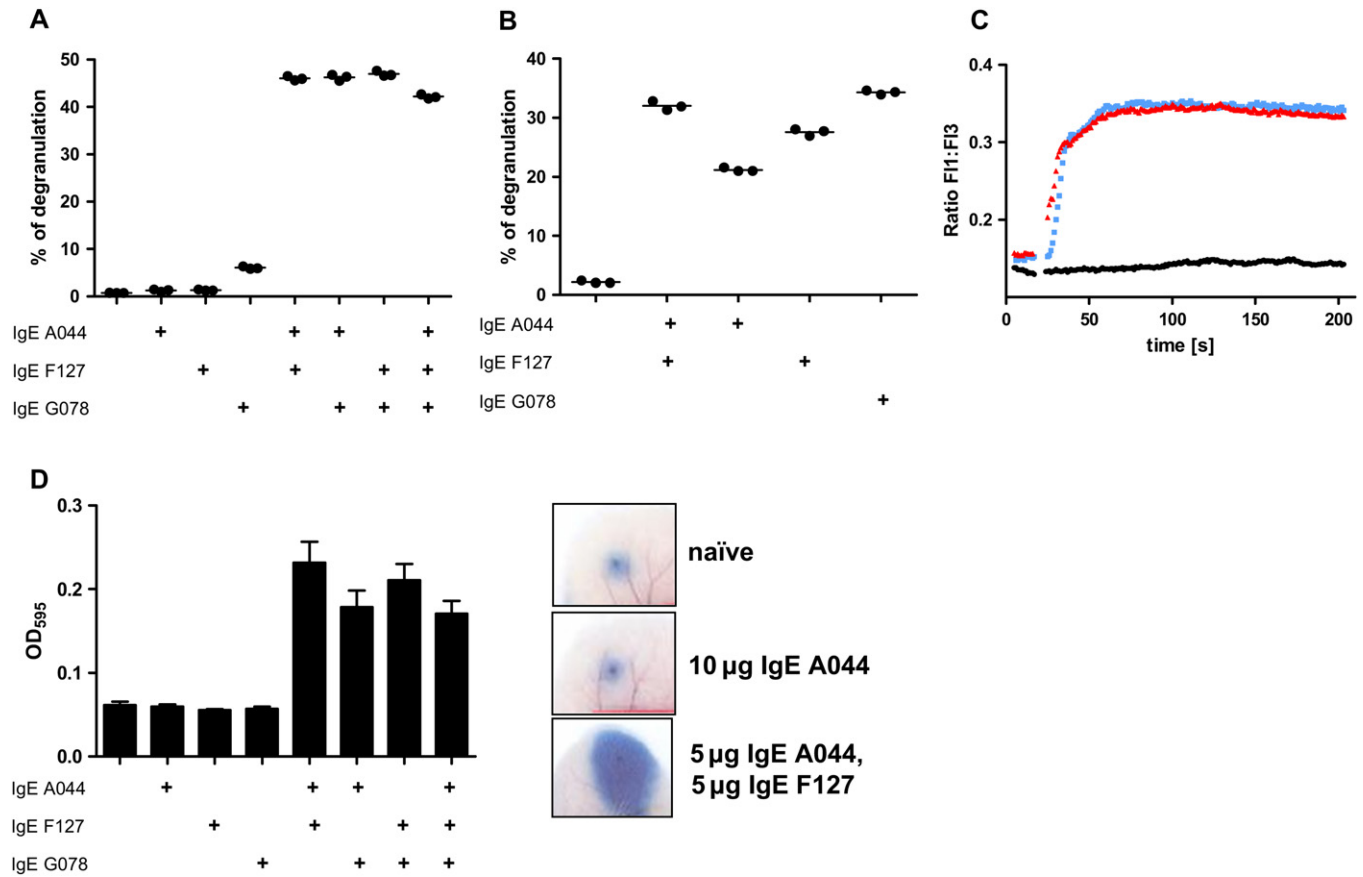


FIG 2. Antibodies that recognize 2 epitopes suffice for BMMC degranulation. Balb/c BMMCs were loaded with 1, 2, or 3 of the indicated IgE clones. Degranulation was induced by monomeric (A) or dimeric (B) rFel d 1. C, Wild-type BMMCs were loaded with IgE A044 (●), IgE A044/F127 (■), or IgE A044/F127/G078 (▲). Calcium flux was induced by addition of monomeric rFel d 1 and was measured by flow cytometry. D, Mice were given intravenous injections of 1, 2, or 3 of the indicated IgE antibodies. Mast cell degranulation was measured by intradermal ear prick test with monomeric rFel d 1. The results show the means $\pm$ SEMs of the OD₅₉₅ data shown are representative for at least 3 independent experiments.

In contrast, preincubating IgE-loaded BMMCs with IgGs did not influence the ability of subsequently added monomeric rFel d 1 to induce BMMC degranulation (data not shown). In accordance with this observation, flow-cytometric analysis showed that IC made from IgG that had been preincubated with rFel d 1, but not free IgG, bound to BMMCs (Fig 3, B). Taken together, these results show that nonblocking, IgG–rFel d 1 ICs block mast cell degranulation.

FcγRIIB is required for inhibition of IgE-mediated mast cell activation

Nonblocking IgGs prevented allergen mediated mast cell degranulation; FcγRIIB expression by mast cells might be involved in this process. To elucidate the mechanisms of IgG-mediated inhibition of mast cell degranulation, BMMCs derived from *FcγRIIB*^{-/-} mice were loaded with IgE antibodies of 2 specificities (A044 and F127) and exposed to IC-comprising monomeric rFel d 1 with the IgG₁ G078 or the IgG_{2a} G078. Interestingly, the rFel d 1–IgG IC failed to block IgE-induced mast cell degranulation in the absence of FcγRIIB (Fig 4, A). Furthermore, preincubation of monomeric rFel d 1 with F(ab)₂ fragments derived from IgG₁ G078 had no influence on the

degranulation of wild-type mast cells loaded with the A044 and F127 IgEs (Fig 4, B). Thus, non blocking IgGs prevent Fel d 1–mediated degranulation in an Fc-dependent and FcγRIIB-dependent manner. To investigate this concept *in vivo*, Balb/c and *FcγRIIB*^{-/-} mice were injected with the IgE antibodies A044 and F127 along with IgG_{2a} G078 or IgG₁ G078. One day later, mice were injected with Evans blue dye, and skin prick tests were performed with monomeric rFel d 1. In accordance with the *in vitro* results, coadministration of IgGs of a third specificity efficiently prevented mast cell degranulation in Balb/c mice (Fig 4, C), whereas no IgG-dependent inhibition of mast cell degranulation was observed in *FcγRIIB*^{-/-} mice. FcγRIIB therefore has an important role in IgG-mediated inhibition of mast cell degranulation under physiological conditions (Fig 4, D). In agreement with the *in vitro* findings, the subclass of IgG antibody used was of minor importance.

Fel d 1–specific IgGs inhibit degranulation of basophils from individuals with cat allergy

We next addressed whether the *in vitro* and *in vivo* findings in mice were relevant to human beings. In individuals with cat allergies, Fel d 1–specific IgEs bind to FcεRI on mast cells and

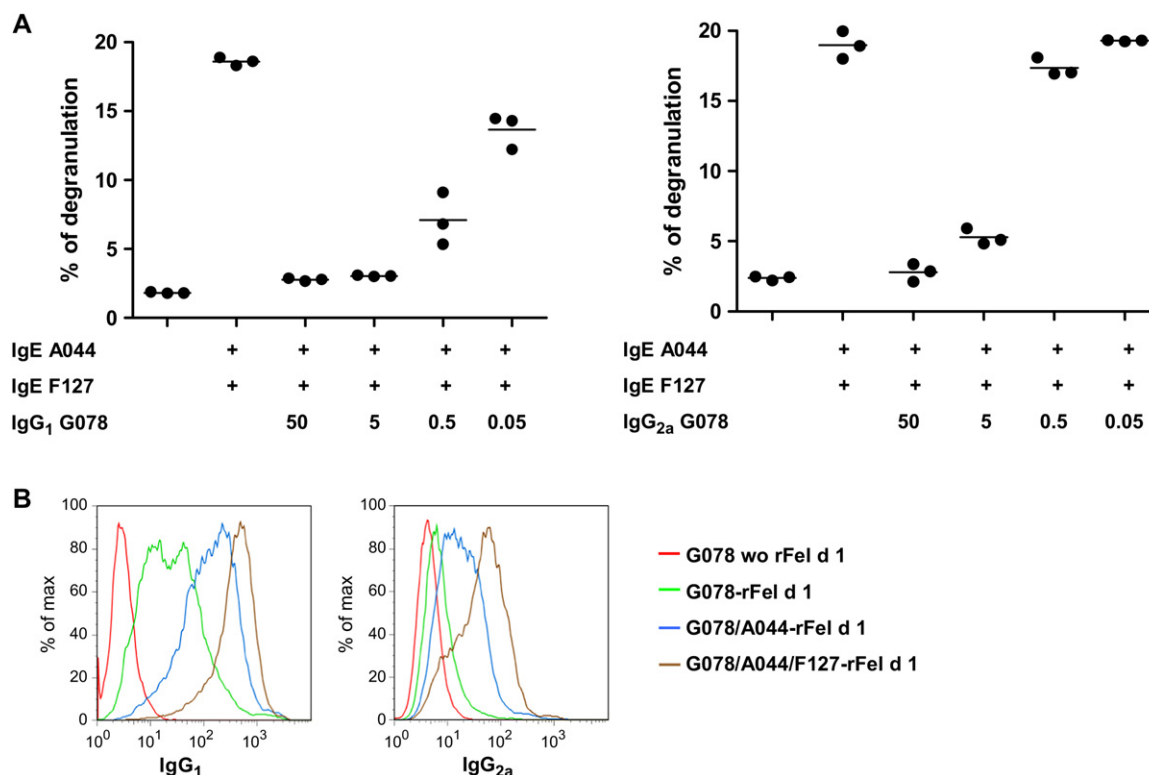


FIG 3. A single IgG can inhibit mast cell degranulation. **A**, Balb/c BMMCs were loaded with IgE A044 and IgE F127 before the addition of monomeric rFel d 1, alone or in a complex with IgG₁ G078 (left) or IgG_{2a} G078 (right). **B**, IgGs bind mast cells only as preformed immune complexes with rFel d 1. Monomeric rFel d 1 was incubated with the indicated IgG clones and loaded on BMMCs. The binding of IgGs was analyzed by flow cytometry. Data shown are representative for 3 or more independent experiments.

basophils, both of which also express FcγRIIB.^{26,27} We generated chimeric antibodies to investigate the influence of Fel d 1–specific IgGs on allergen-induced degranulation in human basophils. We made antibodies that contained the variable regions of the mouse clones A044, F127, and G078 and the constant regions of human IgG₁ and IgG₄ and tested their ability to inhibit Fel d 1–induced degranulation of basophils from subjects with cat allergy. All 3 chimeric mAbs inhibited monomeric rFel d 1–induced degranulation of basophils in a dose-dependent manner (Fig 5, *A and B*, and not shown). As we observed in mice, inhibition of degranulation was independent of the IgG subclass used (Fig 5, *A–D*). Interestingly, a combination of 2 or 3 different IgGs had a greater inhibitory affect on allergen-mediated degranulation than of either IgG alone (Fig 5, *C and D*, and not shown). The increased inhibitory activity of several antibodies might result from stronger cross-linking of FcγRIIB by higher-order allergen-antibody complexes. A single IgG antibody was more efficient in inhibiting allergen-mediated degranulation when a dimeric form of rFel d 1 was used (Fig 5, *E*), supporting this hypothesis. Our data demonstrate that induction of IgGs against a single allergen epitopes is sufficient to induce IgGs that block mast cell activation by polyclonal IgEs.

DISCUSSION

Although the clinical efficacy of SIT is well documented,²⁸ its mechanism is poorly understood. Mast cell and basophil activities, which regulate degranulation and systemic anaphylactic responses, decrease soon after the first injections of allergen.²⁹

Peripheral allergen-specific T-cell activities have been reported to change at later time points of SIT. An important change in T-cell responsiveness has been reported to be caused by the induction of allergen-specific regulatory T cells, which inhibit proliferative and cytokine responses against the allergen.³⁰ SIT also induces production of allergen-specific IgGs. However, the importance of IgG induction in the clinical efficacy of SIT is controversial. Some clinical studies demonstrate a correlation of serum concentrations of allergen-specific IgGs with clinical improvement, but others did not find a correlation.^{31,32}

We previously showed in preclinical studies that induction of allergen-specific IgGs suppresses mast cell degranulation *in vitro* and *in vivo*.¹⁹ To study further the mechanism of IgG-mediated inhibition of mast cell degranulation, we generated a panel of mAbs specific for the major cat allergen Fel d 1. Because the clinical efficacy of SIT is reportedly correlated with induction of allergen-specific IgG₄, we expressed the Fel d 1–specific mAbs as mouse IgE, IgG₁, and IgG_{2a} and as human IgG₁ and IgG₄.

Combinations of 2 Fel d 1–specific IgEs, which recognized different nonoverlapping epitopes on the allergen, induced full mast cell activation; addition of a third IgE with specificity for an additional epitope did not increase degranulation. Conversely, allergen dimers activated mast cells that were loaded with a single IgE, in agreement with the finding that oligomerized allergen with a single monoclonal IgE can induce mast cell activation.³³ This demonstrates that strings of allergen-IgE complexes are sufficient for full mast cell activation—large immune complexes are not required.

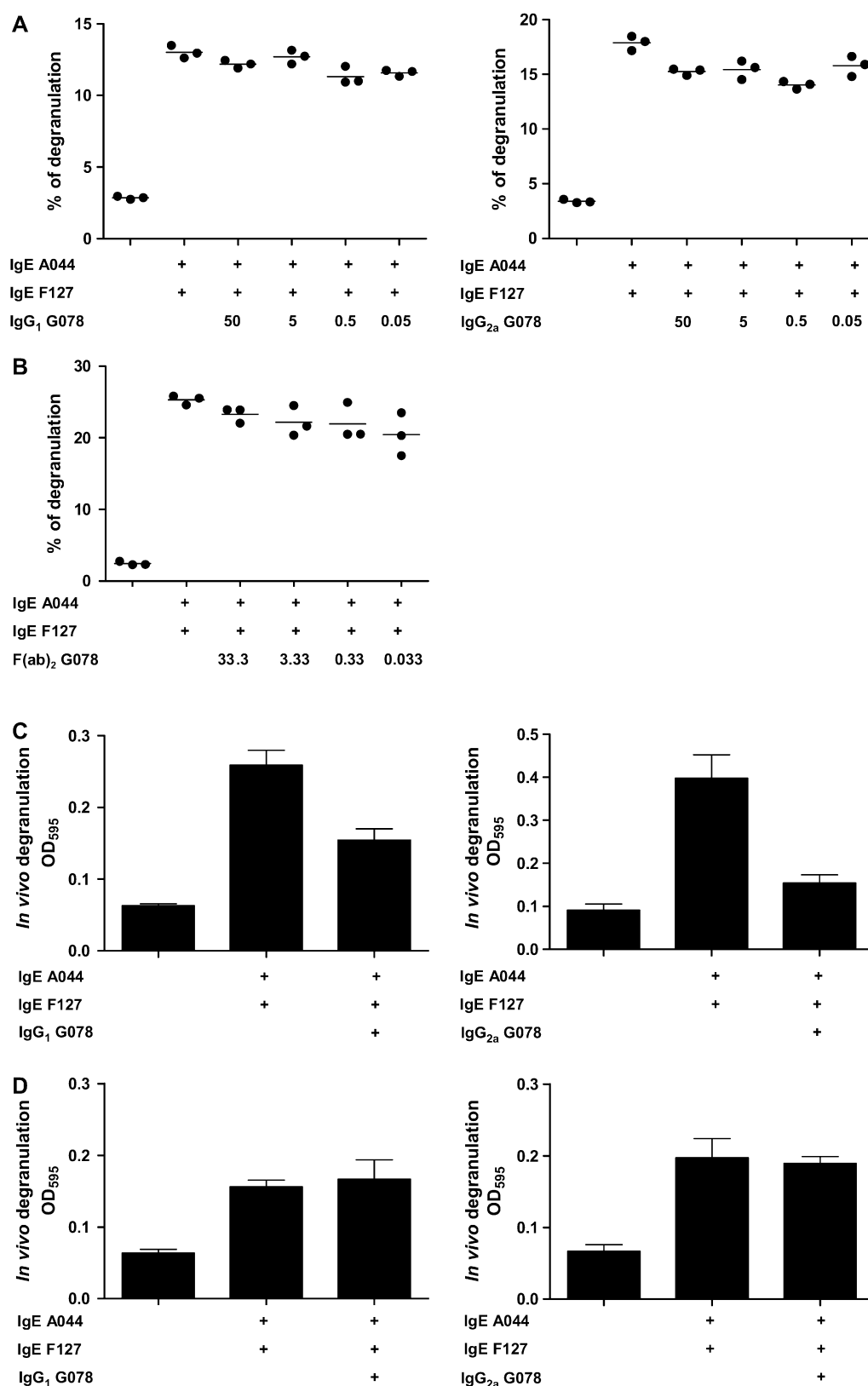


FIG 4. IgGs inhibit mast cell activation in a *FcγRIIB*-dependent fashion. **A**, *FcγRIIB*^{-/-} BMMCs were loaded with IgE A044 and IgE F127. Degranulation was induced by incubation either with monomeric rFcl d 1 alone or in a complex with IgG₁ G078 (left) or IgG_{2a} G078 (right). **B**, Balb/c BMMCs were loaded with IgE A044 and IgE F127. Degranulation was induced by incubation either with monomeric rFcl d 1 alone or complexed with F(ab)₂-G078. Balb/c (C) or *FcγRIIB*^{-/-} (D) mice were given intravenous injections of IgE A044, IgE-F127, and IgG₁ G078 or IgG_{2a} G078. Mast cell degranulation was measured after an intradermal ear prick with monomeric rFcl d 1. The results indicate the means ± SEMs, and data shown are representative for 3 independent experiments.

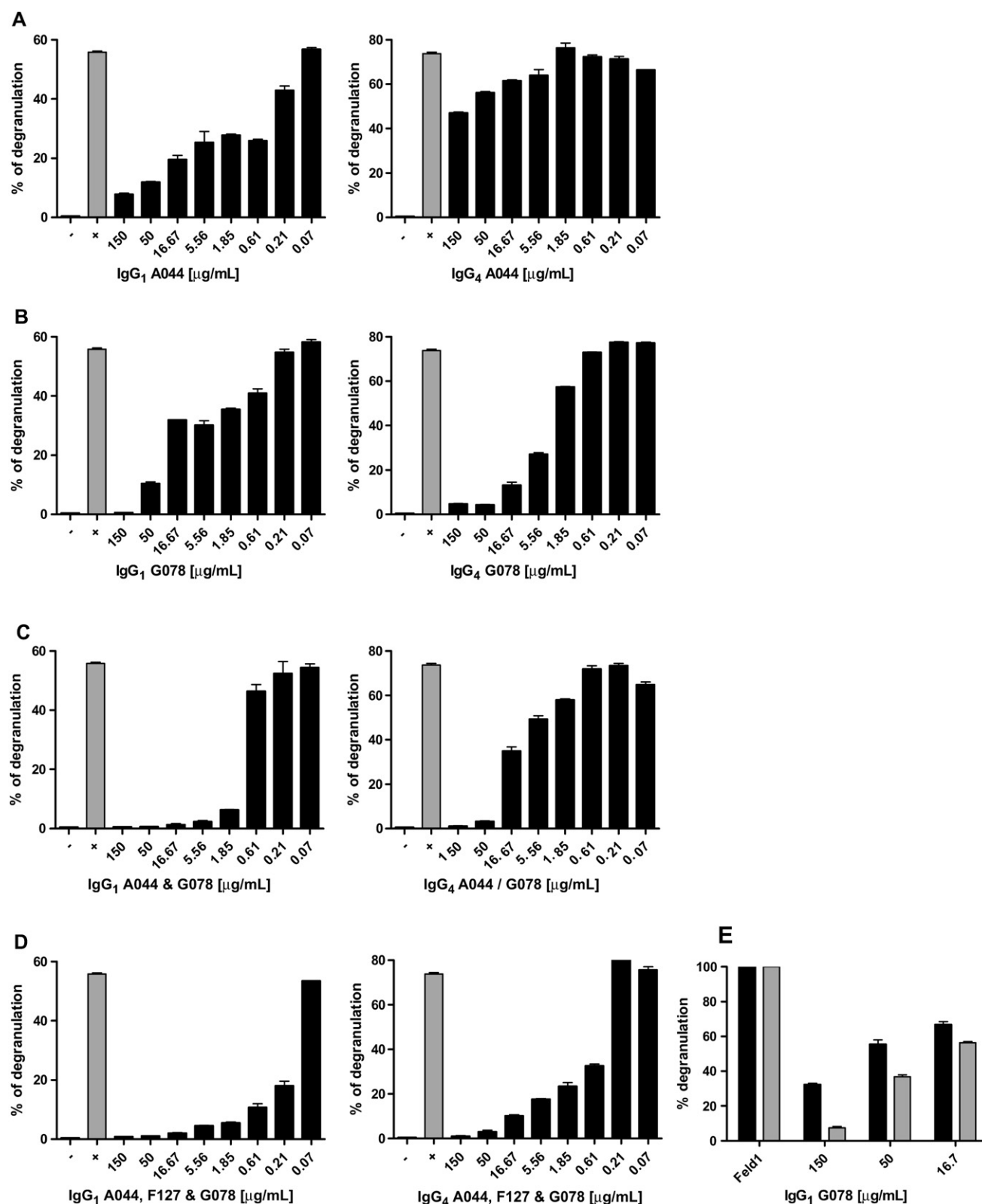


FIG 5. A single IgG can inhibit basophil degranulation of cat allergic individuals. **A-D**, Heparinized whole blood from individuals with cat allergy was incubated with monomeric rFel d 1 (gray bars) or with indicated clones of IgG-rFel d 1 (black bars) IC (left, A-D) or IgG4-rFel d 1 IC (black bars; right panels, A-D). Enlargement of the IC by adding 2 (C) or 3 (D) different IgG₁ or IgG₄ clones (labelled below the respective graphs) increased the inhibition. The results show the means $\pm$ SEMs, and data are representative for 3 donors with cat allergies. **E**, Basophil degranulation was induced by incubation with IC of IgG₁-G078 and monomeric (black bars) or dimeric (gray bars) rFel d 1. The values of the y-axis were normalized to 100% activation. One hundred percent activation was defined by basophils that had been challenged with monomeric rFel d 1. The results show the means $\pm$ SEMs; data shown are derived from 2 independent experiments.

IgE-mediated mast cell activation could be inhibited in a dose-dependent manner by the addition of a monoclonal IgG specific for Fel d 1. Interestingly, IgG subclass did not affect inhibition of the IgE-mediated mast cell activation. This is in contrast to the clinical observation that IgG₄ seems to be most predictive for efficacy of SIT.³⁴ A possible explanation for this discrepancy might be that IgG₄ reflects the antibodies that are produced *de novo* during SIT. Monoclonal IgGs that recognize an epitope on Fel d 1 that does not overlap with the epitopes recognized by the IgEs used for mast cell sensitization were able to block mast cell activation *in vitro* and *in vivo*. This observation indicates that IgG-mediated inhibition of mast cell activation could occur in the absence of competition for epitope binding. Mast cell inhibition could be increased by addition of IgGs that recognize the same epitopes as the IgEs used for mast cell sensitization. Along a similar line, degranulation of basophils from patients with Fel d 1 allergy could be inhibited by single monoclonal IgGs. However, a mixture of IgGs that recognize several epitopes worked better. The fact that IgGs with epitope specificities that differed from those of the IgEs were able to block mast cell activation indicates that in addition to epitope competition between IgGs and IgEs, IgGs can deliver a negative signal to mast cells.

To determine whether this is the case, we studied mast cells that lack the inhibitory FcγRIIB receptor. IgG antibodies of a third specificity failed to block degranulation of these mast cells, demonstrating that IgGs block mast cell activation through activation of FcγRIIB. Interestingly, however, addition of IgGs that share specificities with IgEs on mast cells blocked cellular activation in the absence of FcγRIIB. IgGs therefore block mast cell activation via inhibitory, FcγRIIB-dependent signals and via direct competition for epitopes. We found that IgG binding to a single epitope on Fel d 1 that does not overlap with the epitopes that bind IgE downregulates mast cell activation and can even inhibit basophil degranulation in individuals with cat allergies; this might explain why induction of IgG and the efficacy of allergen immunotherapy do not always correlate.³⁵ These findings might also explain why allergens that have been chemically altered to destroy IgE binding (allergoids) are effective in immunotherapy³⁶; these allergens have not only epitopes that are recognized by T cells but also epitopes that are recognized by IgGs and not IgEs, which block mast cell activation via engagement of FcγRIIB.

These findings could lead to the rational design of safe, epitope-based vaccines for the treatment of allergies. Our data indicate that an allergy vaccine containing a single epitope per allergen might induce protective IgGs. Because single-allergen epitopes cannot trigger mast cell activation, this design would potentially avoid the adverse events associated with allergen immunotherapy.⁵ Vaccines based on single allergen epitopes might be particularly attractive for patients with cat or house dust mite allergies; 90% to 97%^{37,38} of individuals with allergy have IgE-mediated responses to Fel d 1 or the major house dust mite allergen Der p 1. Our data demonstrate that induction of allergen-specific IgGs is a promising strategy to treat common allergies and that induction of IgGs against single epitopes is sufficient to block mast cell activation by polyclonal IgEs.

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