On the role of allergen-specific IgG subclasses for blocking human basophil activation

Monique Vogel[1], Franziska Thoms[2], Gilles Augusto[1], and Martin Bachmann[1]

[1]Department of Immunology University Clinic of Rheumatology and Immunology Inselspital University of Bern Bern Switzerland
[2]Saiba Animal Health Bahnhofstr 13 8808 Pfäffikon Switzerland

March 07, 2024

Abstract

Background: Successful treatment of IgE mediated allergies by allergen-specific immunotherapy (AIT) usually correlates with the induction of allergen-specific IgG4. However, it is not clear whether IgG4 prevents the allergic reaction more efficiently than other IgG subclasses. Here we aimed to compare allergen-specific monoclonal IgG1 and IgG4 antibodies in their capacity to inhibit type I allergic reactions by engaging FcγRIIb. **Methods:** Three monoclonal antibodies (F127, A044 and G078) against Fel d 1, the major cat allergen in humans, recognizing three non-overlapping epitopes of Fel d 1 were tested for their capacity to block human basophils activation in vitro. The affinity of the three monoclonal antibodies in IgG1 and IgG4 formats to FcgRIIb were investigated by Biolayer Interferometry. **Results:** We found that IgG1, which is the dominant subclass induced by viruses, binds with a similar affinity to the FcγRIIb as IgG4 and is comparable at blocking human basophil activation from allergic patients; both by neutralizing the allergen as well as engaging the inhibitory receptor FcγRIIb. **Conclusion:** Hence, the IgG subclass plays a limited role for the protective efficacy of AIT even if IgG4 is considered the best correlate of protection because it is the dominant subclass induced by classical AITs.

1 INTRODUCTION

The World Health Organization (WHO) identifies chronic respiratory diseases including allergies and chronic rhinosinusitis as one of the four major diseases affecting the world's population[1]. According to the ARIA initiative (Allergic Rhinitis and its Impact on Asthma), IgE-mediated inflammation of the nasal mucosa defines allergic rhinitis and causes the respective symptoms upon allergen exposure[2]. Allergies are characterized by two phases: sensitization causing development of allergen-specific memory Th2 and B cells as well as the production of allergen -specific IgE at the early stage and effector functions associated with tissue inflammation and damage at later stages. Patients suffering from allergies usually treat their symptoms with antihistamines or corticosteroids or try to reduce allergen exposure by their avoidance and use of high-efficiency particulate air (HEPA) filters or temperature-controlled laminar airflows. However, for actual treatment of type I allergies, such as allergies against pollen, house dust mite, pet dander, food, or venom toxins, allergen-specific immunotherapy (AIT) represents the only disease modifying option addressing the cause of the illness[2-5]. Commonly, allergens are administered subcutaneously or sublingually but also novel routes such as epicutaneous and intra-lymphatic have also been established during AIT[6-12]. AIT usually requires numerous allergen applications over 3-5 years[13,14]. The main disadvantages are the duration of the therapy and the risk of severe side effect such as anaphylactic reactions. However, successful AIT induces immune tolerance to allergens and affects rhino-conjunctival symptoms and improves asthmatic conditions. AIT does not only mediate short-term but also long-term protection and is qualified as disease-modifying therapy leading overall reduced allergy disease severity, reduced less drug consumption and prevention of future allergen sensitization[2-5,15-19]. In addition to novel routes of allergen administration, clinical testing of

AIT strives for safer and more efficient therapy conditions including use of allergens or allergoids formulated with stronger adjuvants[20-25]or use of peptides rather than full-length allergens[26-29] In addition, we have recently proposed that allergens displayed on virus-like particles may be a safe and efficacious alternative to standard AIT protocols[26,30,31].

The therapeutic mechanisms of AIT still remain a matter of debate. While some argue that induction of allergen-specific IgG antibodies is the key, as they can both neutralize the allergen and engage the inhibitory receptor FcγRIIβ[32], others prefer the hypothesis that regulatory T cells are the masters to keep mast cells and basophils at bay and promote the production of IgG antibodies by altered cytokine secretion[33]. Recently, it has been demonstrated in mice [30]and humans that monoclonal antibodies against Fel d 1, the major cat allergen, can abrogate cat allergy, clearly indicating that anti-allergen IgG antibodies can reduce allergic symptoms[34]. Furthermore, it is clear that successful immunotherapy correlates with induction of allergen-specific IgG4[35]. This has led to the general consensus in the field that induction of IgG4 is the major goal of specific immunotherapy. Others, however, have argued that dominant induction of IgG4 merely reflects the way AIT is performed, namely by multiple injections of small amounts of allergen formulated in Alum and does not reflect a superior efficacy of IgG4 at neutralizing allergens or engaging FcγRIIβ[12,36]. In addition to IgG subclasses, affinities of the antibodies are also important, at least for neutralizing antibodies; the affinity antibodies engaging the FcγRIIβ has been shown to be less demanding[37]. Here we demonstrate that IgG4 binds to FcγRIIβ with similar efficiency as IgG1 and inhibits basophil activation with equivalent efficacy both via the FcγRIIβ as well as the allergen-neutralization pathway. Hence, IgG subclasses play a limited role in the efficacy of AIT.

2 METHODS

2.1 Ethics statement

The experiments involving human blood from cat-allergic and non-cat allergic patients was approved by the KEK (Zurich, Switzerland) ethics committee by the license ZU-Basophils-001 and by the KEK (Bern, Switzerland) ethics committee by the license 00699.

2.2 Production of recombinant Fel d 1 protein

The expression, production, and purification of recombinant Fel d 1 dimer was performed as described elsewhere [38].

2.3 Generation of mAbs against recombinant Fel d 1

By using mammalian cell display [39], isolation and generation of 3 mAbs recognizing the non-overlapping epitopes A044, F127, and G078 on Fel d 1 were recently described [40].

2.4 Basophil activation test with blood from cat-allergic and non-allergic subjects

Whole blood samples were collected in EDTA tubes no more than 18 hours prior testing. Degranulation of basophils was determined using the FLOW CAST Basophil Activation Test (Bühlmann Laboratories, Schönenbuch, Switzerland) following the producer's instructions.

Samples from non-allergic subjects were primed with 1 μg/ml IgE for 1.5 hours at 30°C followed by centrifugation at 400xg for 5 min. The supernatant was discarded to remove the unbound IgE Abs, with the cells subsequently washed twice and resuspended in stimulation buffer following the producer's instructions of the FLOW CAST protocol.

2.5 IgG1 and IgG4 kinetics by Bio-Layer interferometry (BLI)

The analysis of binding kinetics of anti-Fel d 1 IgG1 and IgG4 monoclonal antibodies (F127, A044 and G078) to FcγRIIb was analyzed by Bio-Layer Interferometry (BLI) using an Octet RED96e (Sartorius) instrument. Briefly, high precision Streptavidin (SAX, Sartorius, Fremont, CA, USA) biosensors were saturated with 2 μg/ml of human recombinant biotinylated FcγRIIb (CD32b) (Sino Biological, Cat No 10259_H27H-BI, Beijing, China) in kinetics assay buffer (PBS, 0.1% BSA, 0.02% Tween 20) for 10 min. Association was

carried out in 300 s, with the IgG serially diluted from 100 to 6.3 nM in 1:2 steps. Finally, dissociation was also performed in 300 s. All proteins were diluted in kinetics buffer (KB), a loaded sensor dipped in BLI assay buffer served as drift control. The resulting curves were aligned to the beginning of association step and a 1:1 global model was applied on the fitting.

2.6 BLI-based binding IgG immune-complexes

A comparison of binding kinetics between IgG or IgG in complex with dimeric Fel d 1 and FcγRIIb (CD32b) was also performed using BLI assays. Briefly, SAX biosensors were saturated with 2 μg/ml of human recombinant biotinylated FcγRIIb (CD32b) followed by association with 25 nM of either monomeric IgG1 and IgG4 F127 or in pre-complexed form with 25 nM Fel d 1 for 300 s. Dissociation was also performed in 300 s in kinetics buffer. Drift control was performed with a loaded sensor dipped in kinetics buffer only. The resulting data were normalized to the highest response, using OriginPro (OriginLab Corporation, Northampton, MA, USA) software analysis

3 RESULTS

3.1 IgG1 and IgG4 block activation of basophils with similar efficiency

We recently have described and characterized three monoclonal antibodies (F127, A044 and G078) against Fel d 1, the major cat allergen in humans, recognizing three non-overlapping epitopes of Fel d 1 (Figure 1A)[39,40]. Those variable regions were cloned in front of the human gamma 1, 4 or epsilon chain backbone to express Fel d 1 specific fully human IgG or IgE antibodies of three specificities and 2 different IgG subclasses. To test the neutralization capacity of the Fel d 1 specific IgG1 and IgG4 antibodies, basophils from 2 different individuals (A and B) suffering from cat allergy were stimulated with recombinantly expressed dimeric Fel d 1 and up-regulation of CD63 was used as a read-out for basophil activation (Figure 2). Basophils of both individuals were activated by Fel d 1 and, most importantly, all three Fel d 1 specific mAbs (G078, A044 and F127) either of the IgG1 or IgG4 subtype were able to block basophil activation with similar efficiency. This inhibition may be a mixture of allergen-neutralization and engagement of FcγRIIβ as basophils from allergic individuals likely carry polyclonal Fel d 1-specific IgE antibodies via FcεRI on their surface. To dissect these 2 possibilities, we loaded basophils from non-allergic individuals with the mAbs used above but expressed as IgE.

3.2 IgG1 and IgG4 similarly neutralize allergen and prevent primed basophils from degranulation

To test the ability of IgG1 and IgG4 to block basophil activation by neutralization (Figure 1B), basophils from non-allergic individuals were loaded with each of one of the IgE antibodies (G078, A044, F127) and challenged with Fel d 1 complexed with IgG antibodies of the same specificity. Figure 3A shows that neutralizing inhibition occurred independent of the IgG subclass, as both IgG1 and IgG4 inhibited basophil activation in a similar manner. This may also have been expected as both IgG1 and IgG4 antibodies share the same variable region for each of the 3 mAb types. Differences in neutralizing capacity therefore would have been somewhat unexpected.

3.3 IγΓ1 ανδ IγΓ4 πρεεντ πριμεδ βασοπηιλς φρομ δεγρανυλατιον βψ ενγαγινγ ΦςγPIIβ

We have previously demonstrated *in vitro* and *in vivo* that the allergic reaction mediated by an anti-Fel d 1 IgE of one specificity can be blocked by a single anti-Fel d 1 IgG mAb of a different specificity[40,41,37]. The mechanism of the inhibition involves the inhibitory FcγRIIβ (Figure 1C). IgE bound on mast cells via the FcεRI can bind to the allergen Fel d 1 formed as complex with IgG. The IgG antibody of the allergen complex may bind to the FcγRIIβ simultaneously, which inhibits the signal cascade of the FcεRI and prevents degranulation. To study the ability of IgG subclasses to drive this process, we performed this experiment to compare IgG1 and IgG4 for their ability to engage the FcγRIIβ. Indeed, both antibody subtypes equally well impeded primed human basophils from degranulation (Figure 3B, D). In addition, while a single IgG-specificity distinct fom the IgE used for priming, was able to block basophil activation, addition of 2 IgGs of different specificities further increased inhibition (Figure 3C, D). Comparison of the inhibition obtained

with IgG1 and IgG4 antibody subclasses show no appreciable overall difference between the subclasses. The only minor exception was basophils primed with IgE A044 and then incubated Fel d 1 immune-complexed with IgG1 or IgG4, where inhibition was greater with IgG1 compared to IgG4. This was, however, not the case when basophils were primed with either IgE F127 or IgE G078 (Figure 3 C, D). Thus, IgG1 is at least as potent as IgG4 at blocking basophil activation.

3.4 IγΓ1 ανδ IγΓ4 βινδ το ηυμαν ΦcγPIIβ ωιτη σιμιλαρ αφφινιτψ

In comparison to other Fcγ receptors, the affinity of IgG antibodies to the FcγRIIβ is low even if immune-complexed to antigens. We nevertheless measured the affinity of IgG1 and IgG4 antibodies to FcγRIIb (CD32b) by Biolayer Interferometry using Octet technology [42]. To this end, biotinylated recombinant human FcγRIIβ was immobilized on streptavidin biosensor before incubation with either F127, G078 and A044 in either IgG1 or IgG4 format. As shown in Figure 4, IgG1 and IgG4 antibodies bound with similar affinity to recombinant FcγRIIβ (Figure 4A and Table 1).

We also investigated the effects of immune complex formation on the binding of IgG1 or IgG4 to FcγRIIb by BLI, performing the association step with IgG1 and IgG4 F127 either in monomeric or in complex form with dimeric Fel d 1 (Figure 4 C). The results showed similar association rate (k_{on}) for monomeric and complexed IgG1 and IgG4 whereas the dissociation rate (k_{off}) was 10 times less for the uncomplexed forms of IgG1 and IgG4 indicating an overall higher binding for IgG immune complexes than for IgGs alone. Hence, antibody subclass may have a limited influence on FcγRIIβ binding in both monomeric and complexed form thereby confirming the results obtained in the above cellular assays.

4 DISCUSSION

AIT is the only disease modifying treatment to treat the cause of allergy. Several immune cells and mediators contribute to various degrees to the severity of the allergic reaction. Mast cells and basophils are effector cells of the early phase that are involved in tissue damage, itching and swelling. However, ablating those cells may be technically difficult and also be potentially dangerous, as they are also involved in the fighting against tick and helminth infections, detoxification of arthropod and reptile venoms, and, as recently shown, preserving cardiac function after myocardial infarction[43-45]. B cells expressing as well as IgE and Th2 cells specific for the respective allergen are therefore potentially better targets. However, allergen-specific lymphocytes do not have a unique characteristic to specifically delete them other than the T cell receptor which is notoriously difficult to target. AIT focusses on rendering the body more tolerant to the allergen by changing the immune environment and induction of IgG antibodies[25,46,47]. The high potential of IgG antibodies to reduce the severity of allergic reactions has been shown in mouse models[30,40,32] and more recently in a clinical study demonstrating that it is possible to treat cat allergy with monoclonal anti-Fel d 1 IgG antibodies[34]. In addition, induction of allergen-specific IgG4 or allergen-specific IgE/IgG4 ratio are considered to be the best correlate of AIT efficacy. However, whether IgG4 is more potent than other IgG subclasses or merely happens to be the preferred IgG subclass induced by classical AIT remis still a matter of debate. In fact, induction of IgG4 during classical AIT may actually reflects to some degree natural allergen exposure as seen in bee keepers who are not allergic to bee venom but have high serum levels of specific IgG4[48,49]. Indeed, inducing B cell responses in the absence of innate stimuli, such as toll-like receptor ligands, may preferentially drive IgG4 responses[12,36].

Nevertheless, the role and importance of the induced IgG subclasses during AIT may have important consequences, since use of modern vaccination regimens such as inclusion of stronger adjuvants or formulation with virus-like particles (VLPs) may favour induction of IgG1 rather than IgG4[30]. Indeed, a clinical study to treat house dust mite with Der p 1 coupled to a VLP induced strong IgG1 instead of IgG4 responses[26]. Another important aspect of the safety of AIT is the availability, standardization, and formulation of allergens. Given the fact that allergens coupled to VLPs do not activate basophils[50], but induce strong IgG responses in compared to other approaches, may render AITs safer and more efficient in future; however, the ability of IgG1 to block the allergic reaction remains an important caveat for such new therapies.

To investigate whether IgG4 was more potent than other IgG subclasses at blocking basophil activation,

we expressed 3 different monoclonal antibodies recognizing distinct epitopes on the allergen Fel d 1 in a human IgG1 and IgG4 format[40]. We compared 1) the ability to neutralize the allergen and block basophil activation, 2) to inhibit basophil activation via engagement of FcγRIIβ, and 3) the ability of the IgG1 and IgG4 subclasses to bind to FcγRIIβ. In all 3 types of assays, IgG1 and IgG4 antibodies showed similar efficacy at blocking basophil activation and engaging FcγRIIβ. Hence, IgG4 does not have preferable characteristics for the treatment of allergy. This may indicate that induction of IgG4 is not a pre-requisite for efficient therapy but that amounts and affinities of total IgG may be more important.

ACKNOWLEDGMENTS

This project was supported by funding of the Swiss National Science Foundation (SNF grant 310030_185114 to M.F. B.) and Saiba Animal Health.

CONFLICTS OF INTEREST

F. Thoms and M. F. Bachmann have a financial relationship with Saiba Animal Health involving stock ownership or payments for research activities. All other authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

M.V., F.T. designed, performed and interpreted experiments. G.S. performed experiment and corrected the manuscript. M.V., F.T. and M.F.B. wrote the manuscript.

ORCID

M. F. Bachmann https://orcid.org/0000-0003-4370-2099

M. Vogel http://orcid.org/ 0000-0002-5219-4033

REFERENCES

1. Hellings PW, Borrelli D, Pietikainen S, et al. European Summit on the Prevention and Self-Management of Chronic Respiratory Diseases: report of the European Union Parliament Summit (29 March 2017). *Clin Transl Allergy.* 2017;7:49.

2. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol.* 1998;102(4 Pt 1):558-562.

3. Calderon MA, Alves B, Jacobson M, Hurwitz B, Sheikh A, Durham S. Allergen injection immunotherapy for seasonal allergic rhinitis.*Cochrane Database Syst Rev.* 2007(1):CD001936.

4. Cox L, Nelson H, Lockey R, et al. Allergen immunotherapy: a practice parameter third update. *J Allergy Clin Immunol.* 2011;127(1 Suppl):S1-55.

5. Wise SK, Schlosser RJ. Subcutaneous and sublingual immunotherapy for allergic rhinitis: what is the evidence? *Am J Rhinol Allergy.*2012;26(1):18-22.

6. Senti G, Graf N, Haug S, et al. Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy. *J Allergy Clin Immunol.* 2009;124(5):997-1002.

7. Senti G, von Moos S, Tay F, et al. Epicutaneous allergen-specific immunotherapy ameliorates grass pollen-induced rhinoconjunctivitis: A double-blind, placebo-controlled dose escalation study. *J Allergy Clin Immunol.* 2012;129(1):128-135.

8. Jutel M, Akdis CA. Novel immunotherapy vaccine development.*Curr Opin Allergy Clin Immunol.* 2014;14(6):557-563.

9. Kundig TM, Johansen P, Bachmann MF, Cardell LO, Senti G. Intralymphatic immunotherapy: time interval between injections is essential. *J Allergy Clin Immunol.* 2014;133(3):930-931.

10. Soyka MB, van de Veen W, Holzmann D, Akdis M, Akdis CA. Scientific foundations of allergen-specific immunotherapy for allergic disease.*Chest.* 2014;146(5):1347-1357.

11. Kundig TM, Klimek L, Schendzielorz P, Renner WA, Senti G, Bachmann MF. Is The Allergen Really Needed in Allergy Immunotherapy? *Curr Treat Options Allergy.* 2015;2(1):72-82.

12. Bachmann MF, Kundig TM. Allergen-specific immunotherapy: is it vaccination against toxins after all? *Allergy.* 2017;72(1):13-23.

13. Cox LS, Larenas Linnemann D, Nolte H, Weldon D, Finegold I, Nelson HS. Sublingual immunotherapy: a comprehensive review. *J Allergy Clin Immunol.* 2006;117(5):1021-1035.

14. Kariyawasam HK, Rotiroti G, Robinson DS. Sublingual immunotherapy in allergic rhinitis: indications, efficacy and safety. *Rhinology.*2013;51(1):9-17.

15. Durham SR, Walker SM, Varga EM, et al. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med.* 1999;341(7):468-475.

16. Pajno GB, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. *Clin Exp Allergy.* 2001;31(9):1392-1397.

17. Eng PA, Reinhold M, Gnehm HP. Long-term efficacy of preseasonal grass pollen immunotherapy in children. *Allergy.*2002;57(4):306-312.

18. Moller C, Dreborg S, Ferdousi HA, et al. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J Allergy Clin Immunol.*2002;109(2):251-256.

19. Jacobsen L, Niggemann B, Dreborg S, et al. Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. *Allergy.* 2007;62(8):943-948.

20. Basomba A, Tabar AI, de Rojas DH, et al. Allergen vaccination with a liposome-encapsulated extract of Dermatophagoides pteronyssinus: a randomized, double-blind, placebo-controlled trial in asthmatic patients. *J Allergy Clin Immunol.* 2002;109(6):943-948.

21. Fonseca DE, Kline JN. Use of CpG oligonucleotides in treatment of asthma and allergic disease. *Adv Drug Deliv Rev.*2009;61(3):256-262.

22. Senti G, Johansen P, Haug S, et al. Use of A-type CpG oligodeoxynucleotides as an adjuvant in allergen-specific immunotherapy in humans: a phase I/IIa clinical trial. *Clin Exp Allergy.*2009;39(4):562-570.

23. Klimek L, Willers J, Hammann-Haenni A, et al. Assessment of clinical efficacy of CYT003-QbG10 in patients with allergic rhinoconjunctivitis: a phase IIb study. *Clin Exp Allergy.* 2011;41(9):1305-1312.

24. Leuthard DS, Duda A, Freiberger SN, et al. Microcrystalline Tyrosine and Aluminum as Adjuvants in Allergen-Specific Immunotherapy Protect from IgE-Mediated Reactivity in Mouse Models and Act Independently of Inflammasome and TLR Signaling. *J Immunol.* 2018;200(9):3151-3159.

25. Zubeldia JM, Ferrer M, Davila I, Justicia JL. Adjuvants in Allergen-Specific Immunotherapy: Modulating and Enhancing the Immune Response. *J Investig Allergol Clin Immunol.* 2019;29(2):103-111.

26. Kundig TM, Senti G, Schnetzler G, et al. Der p 1 peptide on virus-like particles is safe and highly immunogenic in healthy adults.*J Allergy Clin Immunol.* 2006;117(6):1470-1476.

27. Worm M, Lee HH, Kleine-Tebbe J, et al. Development and preliminary clinical evaluation of a peptide immunotherapy vaccine for cat allergy.*J Allergy Clin Immunol.* 2011;127(1):89-97, 97 e81-14.

28. Patel D, Couroux P, Hickey P, et al. Fel d 1-derived peptide antigen desensitization shows a persistent treatment effect 1 year after the start of dosing: a randomized, placebo-controlled study. *J Allergy Clin Immunol.* 2013;131(1):103-109 e101-107.

29. Focke-Tejkl M, Weber M, Niespodziana K, et al. Development and characterization of a recombinant, hypoallergenic, peptide-based vaccine for grass pollen allergy. *J Allergy Clin Immunol.*2015;135(5):1207-1207 e1201-1211.

30. Schmitz N, Dietmeier K, Bauer M, et al. Displaying Fel d1 on virus-like particles prevents reactogenicity despite greatly enhanced immunogenicity: a novel therapy for cat allergy. *J Exp Med.*2009;206(9):1941-1955.

31. Engeroff P, Caviezel F, Storni F, Thoms F, Vogel M, Bachmann MF. Allergens displayed on virus-like particles are highly immunogenic but fail to activate human mast cells. *Allergy.* 2018;73(2):341-349.

32. Shamji MH, Valenta R, Jardetzky T, et al. The role of allergen-specific IgE, IgG and IgA in allergic disease. *Allergy.*2021.

33. Celebi Sozener Z, Mungan D, Cevhertas L, Ogulur I, Akdis M, Akdis C. Tolerance mechanisms in allergen immunotherapy. *Curr Opin Allergy Clin Immunol.* 2020;20(6):591-601.

34. Orengo JM, Radin AR, Kamat V, et al. Treating cat allergy with monoclonal IgG antibodies that bind allergen and prevent IgE engagement.*Nat Commun.* 2018;9(1):1421.

35. Nakagome K, Nagata M. Allergen Immunotherapy in Asthma.*Pathogens.* 2021;10(11).

36. Bachmann MF, Mohsen MO, Kramer MF, Heath MD. Vaccination against Allergy: A Paradigm Shift? *Trends Mol Med.* 2020;26(4):357-368.

37. Zha L, Leoratti FMS, He L, et al. An unexpected protective role of low-affinity allergen-specific IgG through the inhibitory receptor FcgammaRIIb. *J Allergy Clin Immunol.* 2018;142(5):1529-1536 e1526.

38. Thoms F, Jennings GT, Maudrich M, et al. Immunization of cats to induce neutralizing antibodies against Fel d 1, the major feline allergen in human subjects. *J Allergy Clin Immunol.*2019;144(1):193-203.

39. Beerli RR, Bauer M, Buser RB, et al. Isolation of human monoclonal antibodies by mammalian cell display. *Proc Natl Acad Sci U S A.*2008;105(38):14336-14341.

40. Uermosi C, Beerli RR, Bauer M, et al. Mechanisms of allergen-specific desensitization. *J Allergy Clin Immunol.*2010;126(2):375-383.

41. Uermosi C, Zabel F, Manolova V, et al. IgG-mediated down-regulation of IgE bound to mast cells: a potential novel mechanism of allergen-specific desensitization. *Allergy.* 2014;69(3):338-347.

42. Petersen RL. Strategies Using Bio-Layer Interferometry Biosensor Technology for Vaccine Research and Development. *Biosensors (Basel).* 2017;7(4).

43. Ngkelo A, Richart A, Kirk JA, et al. Mast cells regulate myofilament calcium sensitization and heart function after myocardial infarction.*J Exp Med.* 2016;213(7):1353-1374.

44. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol.* 2010;125(2 Suppl 2):S73-80.

45. Cildir G, Pant H, Lopez AF, Tergaonkar V. The transcriptional program, functional heterogeneity, and clinical targeting of mast cells.*J Exp Med.* 2017;214(9):2491-2506.

46. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy.*J Allergy Clin Immunol.* 2007;119(4):780-791.

47. Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited.*Curr Opin Allergy Clin Immunol.* 2004;4(4):313-318.

48. Nittner-Marszalska M, Malolepszy J, Zak-Nejmark T. [Skin tests, total IgE and venom specific IgE and IgG4 in serum of bee-keepers].*Pneumonol Alergol Pol.* 1993;61(7-8):342-345.

49. Varga EM, Kausar F, Aberer W, et al. Tolerant beekeepers display venom-specific functional IgG4 antibodies in the absence of specific IgE. *J Allergy Clin Immunol.* 2013;131(5):1419-1421.

50. Engeroff P, Caviezel F, Storni F, Thoms F, Vogel M, Bachmann MF. Allergens displayed on virus-like particles are highly immunogenic but fail to activate human mast cells. *Allergy.* 2017.

Table 1: Kinetic parameters for the IgG1/IgG4 – CD32b interaction calculated by BLI

Analyte	**K_D [M]**	**k_{on} [$M^{-1}s^{-1}$]**	**k_{off} [s^{-1}]**	**R^2**
IgG1 F127	2.7×10^{-8}	6.0×10^{4}	1.6×10^{-3}	0.9950
IgG1 G078	2.8×10^{-8}	8.2×10^{4}	1.2×10^{-3}	0.9862
IgG1 A044	1.8×10^{-8}	4.5×10^{4}	8.1×10^{-4}	0.9980
IgG4 F127	2.7×10^{-8}	5.1×10^{4}	1.4×10^{-3}	0.9952
IgG4 G078	3.6×10^{-8}	2.3×10^{4}	1.2×10^{-3}	0.9980
IgG4 A044	3.4×10^{-8}	4.8×10^{4}	1.6×10^{-3}	0.9975

FIGURE LEGENDS

Figure 1. Binding and protective role of allergen-specific IgG antibodies in mast cell degranulation. A) Mice were immunized with the vaccine Fel d 1 coupled to Qβ-VLP. By mammalian cell display, three monoclonal antibodies (G078, A044, F127) were cloned in IgG and IgE formats that recognize non-overlapping B cell epitopes. B) Anti-Fel d 1 IgG antibody (e.g. F127) having the same epitope specificity as anti-Fel d 1 IgE (e.g. F127) neutralizes Fel d 1. C) Anti-Fel d 1 IgG antibody (e.g. F127) having a different specificity as anti-Fel d 1 IgE (e.g. G078) inhibit degranulation via FcγRIIb.

Figure 2. IgG 1 and IgG4 mAbs equally well prevent basophil activation from cat allergic subjects. Blood from two (A and B) cat allergic subjects were collected and incubated with the amount of recombinant Fel d 1 (subject A: 0.015 nM; subject B: 0.022 nM) that causes 50% of their basophils to degranulate. That respective amount of Fel d 1 was also pre-mixed with 3, 30, and 300 nM of Fel d 1 specific mAbs either in the format of IgG1 or IgG4. The percentage of CD63+ within the CCR3+ basophil population was measured.

Φιγυρε 3. ΙγΓ1 ανδ ΙγΓ4 πρεεντ δεγρανυλατιον βψ νευτραλιζατιον ανδ σιγναλλινγ ια τηε ινηιβιτορψ ρεζεπτορ ΦζγΡΙΙβ. Blood from two non-allergic subjects was collected and primed with Fel d 1 specific IgE. A) Basophils were primed either with IgE A044, F127 or G078. Primed basophils were treated with recombinant Fel d 1 alone or pre-mixed with the same epitope expressed as IgG1 or IgG4. B) Basophils were primed either with IgE A044, F127 or G078. Primed basophils were treated with recombinant Fel d 1 alone or pre-mixed with the IgG1 or IgG4 antibodies exhibiting the other two epitopes than the one used for priming. C) Basophils were primed either with IgE A044, F127 or G078. Primed basophils were treated with recombinant Fel d 1 alone or pre-mixed with a combination of two IgG1 or IgG4 antibodies exhibiting the other two epitopes than the one used for priming. D) % inhibition of basophil activation when basophils were primed either with IgE A044, F127 or G078 and treated with Fel d 1 pre-mixed with either IgG1 or IgG4 antibodies.

Φιγυρε 4. ΒΛΙ σενσοργραμς ιλλυστρατινγ τηε ιντεραςτιον ΙγΓ1 ανδ ΙγΓ4 αντιβοδιες το ΦζγΡΙΙβ. A) Binding of monomeric F127, G078 and A044 in either IgG1 or IgG4 format. B) Binding of IgG1 and IgG4 F127 either as monomer or in pre-complexed form with Fel d 1. The on-rate (k_{on}) and off-rate (k_{off}) constants were compared. In both assays association and dissociation were performed 300s.

Figure 1

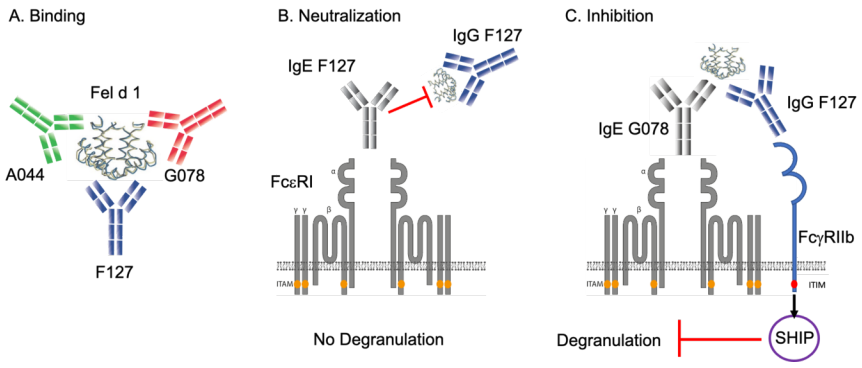


Figure 2

A B

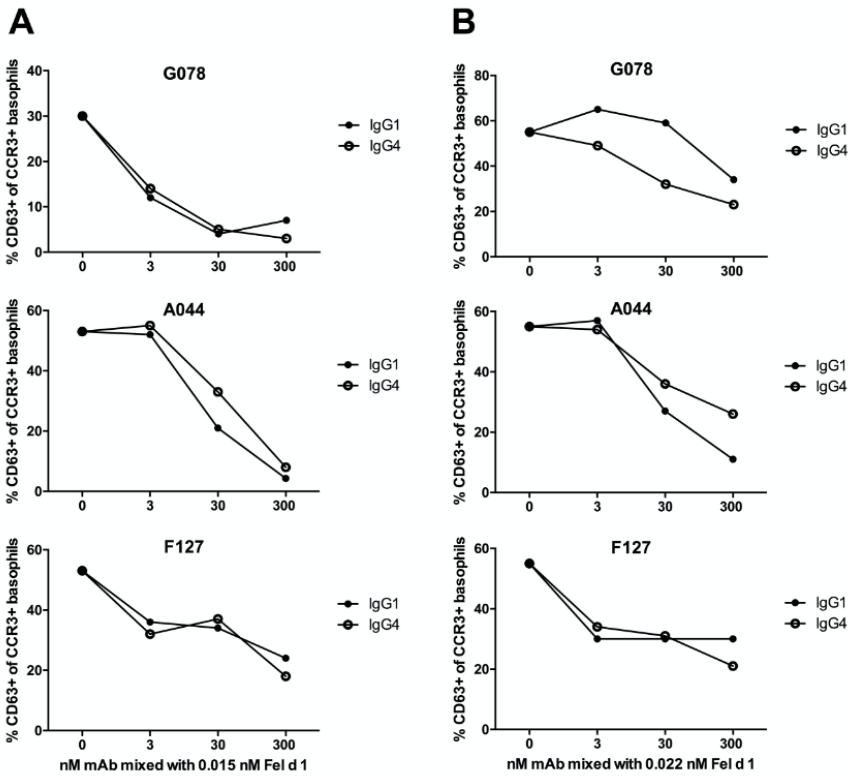


Figure 3

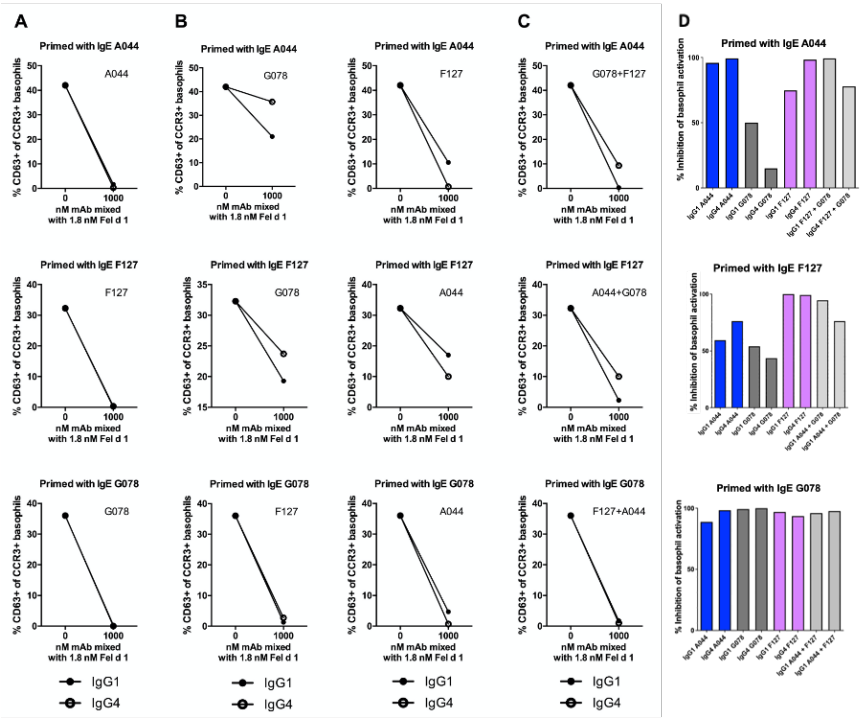
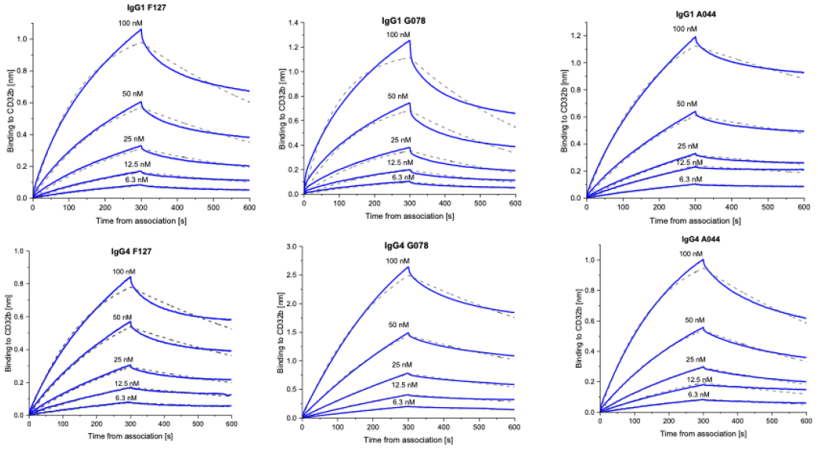


Figure 4

A



B