HHS Public Access

Author manuscript

J Allergy Clin Immunol. Author manuscript; available in PMC 2017 April 01.

Published in final edited form as:

J Allergy Clin Immunol. 2016 April ; 137(4): 1256–1258.e11. doi:10.1016/j.jaci.2015.10.043.

Parameters Determining the Efficacy of CD32 to Inhibit Activation of FcεRI in Human Basophils

Donald MacGlashan Jr., MD, PhD and **Robert G. Hamilton, PhD**

Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Baltimore, MD 21224

Capsule Summary

The conditions needed to readily observe the role of CD32 in modulating IgE-mediated secretion are enumerated and include consideration of IgG subclass, with a possible emphasis on IgG3, antibody:antigen ratios, but with no evidence that polymorphic variants of CD32 influence its function.

Keywords

IgE; histamine release; chimeric antibodies

To the editor:

The mechanisms that underlie the success of allergen immunotherapy are varied and the precise details remain unclear. Immune deviation and blocking antibodies are two important candidate explanations and under the category of blocking antibodies are two often discussed possibilities; 1) simple competition for allergen between IgG antibodies developed during immunotherapy and IgE antibodies on the mast cell and basophil cell surface (what we are calling stoichiometric blockade) or 2) interaction between IgG antibodies and CD32 (a low affinity IgG receptor, FcgRII) on mast cells [1, 2] or basophils [3-6] leading to inhibition of the IgE-mediated response. There is conflicting information about the role of CD32 in this reaction in humans. One possible issue is whether human mast cells even express CD32b, the inhibitory IgG receptor. Other issues relate to the relative ability of different IgG subclasses to interact with CD32b or CD32a [7] and whether CD32a, normally considered an activating IgG receptor, acts in an inhibitory capacity in the context of CD32b or cell type [4, 5]. Human basophils express both CD32a and CD32b [3-6] and it has been clearly demonstrated that CD32 can mediate inhibition of the IgE-dependent reaction. But there are a variety of studies that have demonstrated that not all IgG subclasses bind to CD32a or b [7]. Also, there are polymorphisms in CD32 that influence binding and/or function to certain subclasses [7, 8]. Furthermore, immunotherapy generates different elevations in IgG subclasses and for a variety of reasons, studies have focused on IgG1 and IgG4 and very infrequently

(dmacglas@jhmi.edu, 410-550-2145), (rhamilto@jhmi.edu, 410-550-2031)

examine IgG2 or IgG3. But binding studies have shown that IgG4 does not interact with CD32 (a or b) [7]. What remains unclear is the relative ability of IgG1, 2 and 3 to interact with CD32 and the potential for polymorphisms to further differentiate binding.

Using partially enriched human basophils (see methods in the online repository) and a series of transfectoma antibodies all utilizing the same CDR specific for nitrophenyl (NP) but varying the heavy chain subclass (IgE, IgG1, 2, 3 and 4), the ability of the various IgG subclasses to inhibit IgE-mediated release from basophils sensitized with NP-specific IgE was examined. Three reaction designs were examined, holding IgG constant and varying antigen (which is presumably that natural situation), holding allergen constant and varying IgG and a third approach presented in the online repository (see also Figure E1 for schematic of the experimental design). Figure 1 shows results using the first two methods. Using the first method, the amount of inhibition by IgG was titrated to approximately 50% in order to detect alteration of the response in either the positive or negative direction when including blockade of CD32 and to not bias the reaction to complete stoichiometric blocking (see online repository). To block CD32 and therefore test the involvement of CD32-mediated inhibition rather than simple stoichiometric blockade, an engineered high affinity anti-CD32b Ab and a commercial anti-CD32a Ab were used. The density of CD32a and CD32b were also monitored by flow cytometry. The results, focusing on the highest concentrations of antigen and antibody, Figure 1, panels A-F, indicate that it was difficult to detect functional interaction with CD32b when IgG1 was used, but IgG2 and IgG3 effectively engaged CD32b (the degree of CD32b involvement was measured by the extent of reversal-of-inhibition when including the CD32b-blocking antibody, Ab10523). At lower concentrations of antigen, only stoichiometric inhibition is observed. Figure E2 (online repository) shows the importance of absolute antigen concentration and the importance of IgG:allergen ratios. In the second design shown in figure 1G (holding antigen constant and varying IgG), it can be again observed that IgG1 did not engage CD32b while IgG2 and IgG3 did. As shown in the online repository, figure E3, IgG4 did not cause inhibition. These results also demonstrated that IgG3 was 10 fold more efficacious in interacting with CD32b than IgG2, such that only 1 IgG3 per 20 antigen molecules was necessary to mediate inhibition while approximately 0.5:1 ratios were needed for IgG2. Figure 1, panels B, D, & F, also examined the ability to further reverse inhibition by the inclusion of CD32a blockade with Ab IV.3 (see methods). There was some further reversal by addition of the Ab IV.3 although the best reversal occurred with CD32b-blockade. As discussed in the online repository, heterogeneity in the relative participation of CD32 was correlated with levels of CD32 expression (Table E1) and reversal of IgG2 and IgG3 were correlated. Furthermore, as shown in figure E4, polymorphisms in CD32a and CD32b did not influence the relative participation of CD32. As a low affinity IgG receptor, CD32 is not thought to engage monomeric IgG effectively but concentrations of IgG in plasma are very high so the ability of nonspecific IgG to inhibit participation of CD32 (using nonspecific IgG (nsIgG) as a substitute for Ab10523) was examined. Figure E5 shows that reversal of CD32 effects occur with an IC50 of 150 μg/ml of nsIgG.

These and our previous studies [6] suggest that there are several important parameters, possibly 5 dimensions, which determine whether it is possible to observe the inhibitory function of CD32 on basophils. These results place some constraints on how to explore the

role of CD32 in the basophil reaction during immunotherapy and the variability in the published literature on this issue might be accounted for by consideration of the constraints. It would appear that attempts to correlate CD32 function with clinical outcome would require a multiple regression analysis that included parameters that could be measured, although some with some difficulty. These include, 1) density of allergen-specific IgE (it is possible to overcome CD32-mediated inhibition with high densities of allergen-specific IgE), 2) the subclass of IgG (with IgG3 >10 fold more potent than IgG2 > IgG1 and no IgG4-mediated inhibition), 3) expression level of CD32, 4) absolute concentration of allergen and 5) the ratio of IgG:allergen. IgG3 aggregates appear extremely efficacious and potent. However, the effect is sensitive to the precise Ab:Ag ratio and the range at which any of the subclasses effectively dominate inhibition by engagement with CD32 is narrow. Outside this window, stoichiometric blocking dominates any observed inhibition. While these are important parameters, our results showing that nonspecific IgG at high enough concentrations can blunt the interaction of antigen-specific IgG aggregates to interact with CD32 raises important questions on how to study blocking antibodies in the context of CD32. The reagents used to block CD32 are an important part of how to explore a role for CD32 but the inclusion of serum or plasma --in order to present the reaction with blocking antibodies-- would appear to be problematic for assessing CD32 function. If nonspecific IgG operates in this manner *in vivo*, it would suggest that either there are other conditions that allow IgG antibodies to interact with basophils in circulation or that this reaction is not relevant in circulation but perhaps relevant in tissues where the concentrations of nonspecific IgG might be more conducive to aggregates regulating this reaction. In addition, for therapeutics that are designed to work with CD32, use of the IgG3 subclass might be a potential improvement. Despite the apparent complexity of the requirements for readily observing a CD32-effect, it is possible to find conditions in which this effect is nearly pure, i.e., inhibition solely due to CD32-mediated inhibition with no stoichiometric blocking. These studies used a single transfectoma series of cloned antibodies and the basis for the differences in subclass behavior with respect to CD32, although observed in other studies of FcgR-subclass affinity [7], are unclear. Further study of natural antibodies is needed but the results do suggest that measurement of IgG2 and IgG3 antibodies is warranted in immunotherapy studies because it is these two subclasses that appear to be the most efficacious in recruiting CD32 into reactions with antigen and antibodies on the human basophil.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Daeron M, Malbec O, Latour S, Arock M, Fridman WH. Regulation of high affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. J. Clin. Invest. 1995; 95:577–85. [PubMed: 7860741]
2. Takai T, Ono M, Hikida M, Ohmori H, Ravetch JV. Augmented humoral and anaphylactic responses in Fc gamma RII-deficient mice. Nature. 1996; 379:346–9. [PubMed: 8552190]

3. Kepley CL, Cambier JC, Morel PA, Lujan D, Ortega E, Wilson BS, et al. Negative regulation of FcepsilonRI signaling by FcgammaRII costimulation in human blood basophils. J Allergy Clin Immunol. 2000; 106:337–48. [PubMed: 10932079]

4. Cady CT, Powell MS, Harbeck RJ, Giclas PC, Murphy JR, Katial RK, et al. IgG antibodies produced during subcutaneous allergen immunotherapy mediate inhibition of basophil activation via a mechanism involving both FcgammaRIIA and FcgammaRIIB. Immunol Lett. 2010; 130:57–65. [PubMed: 20004689]

5. Cassard L, Jonsson F, Arnaud S, Daeron M. Fcgamma receptors inhibit mouse and human basophil activation. J Immunol. 2012; 189:2995–3006. [PubMed: 22908332]

6. Macglashan D Jr. Moore G, Muchhal U. Regulation of IgE-mediated signalling in human basophils by CD32b and its role in Syk down-regulation: basic mechanisms in allergic disease. Clin Exp Allergy. 2014; 44:713–23. [PubMed: 24734927]

7. Warmerdam PA, van de Winkel JG, Vlug A, Westerdaal NA, Capel PJ. A single amino acid in the second Ig-like domain of the human Fc gamma receptor II is critical for human IgG2 binding. J Immunol. 1991; 147:1338–43. [PubMed: 1831223]

8. Li X, Wu J, Carter RH, Edberg JC, Su K, Cooper GS, et al. A novel polymorphism in the Fcgamma receptor IIB (CD32B) transmembrane region alters receptor signaling. Arthritis Rheum. 2003; 48:3242–52. [PubMed: 14613290]

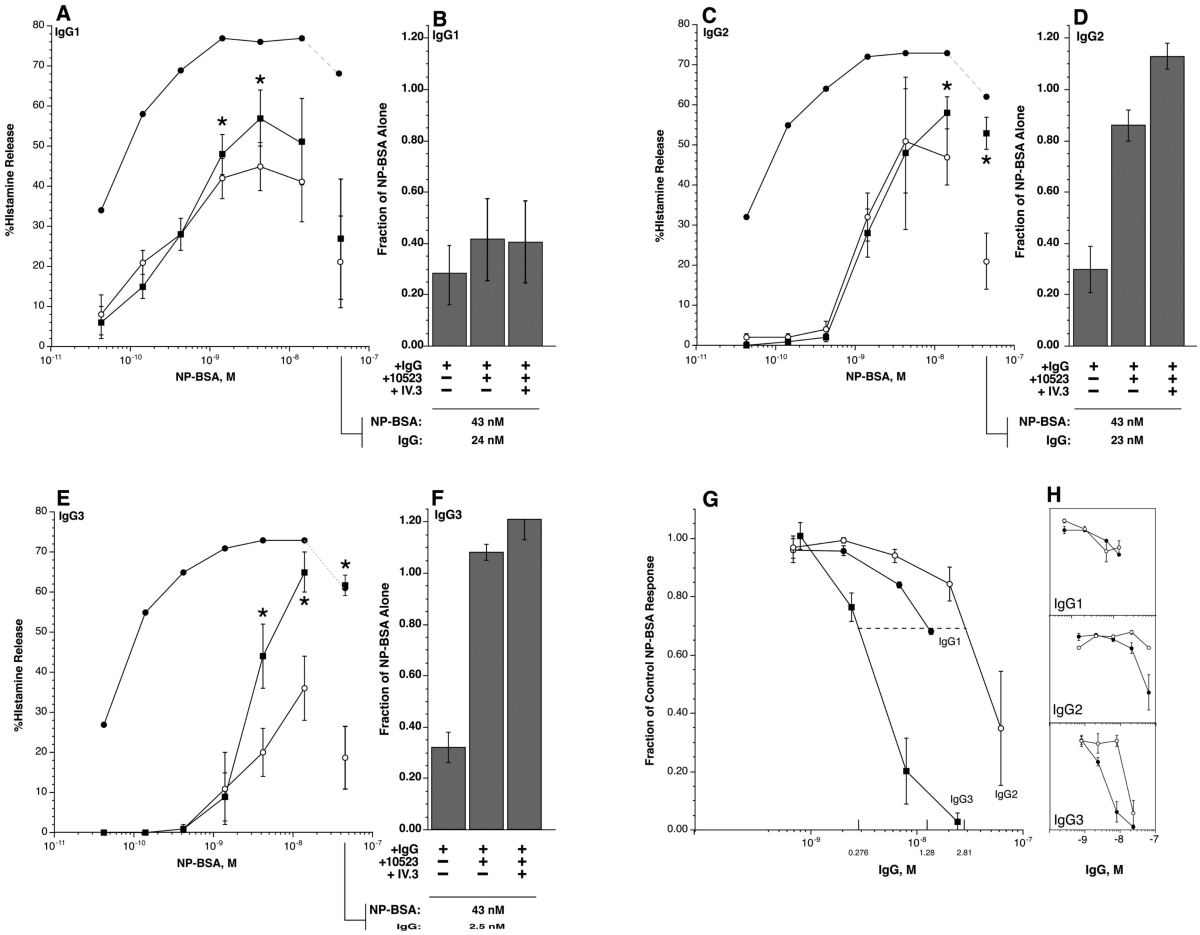

Figure 1.

Inhibition of antigen-induced release by human IgG subclass antibodies. Each panel examines one of the three human IgG subclass anti-NP antibodies using the protocol described in the text and in the online repository. The plots in panels A, C, and E are a composite of two reaction conditions with different replication numbers. At 43 nM (3 μg/ml) NP-BSA, 3 times as much antibody was used as the next highest concentration of NP-BSA, 14 nM (1 μg/ml) (to maintain the same Ab:Ag ratio). Otherwise, the concentration of subclass antibody was held constant for all NP-BSA concentrations ≤ 14 nM. For IgG1 (panel A), the Ab concentrations were 24 nM (n=2) and 8 nM (n=5), for IgG2 (panel B), the Ab concentrations were 23 nM (n=13) and 7.7 nM (n=7), for IgG3 (panel C) the Ab concentrations were 2.5 nM (n=13) and 0.83 nM (n=8). For each panel, (●) response to NP-BSA without subclass antibody, (○) response with subclass antibody, (■) response with subclass antibody and Ab10523 at 50 μg/ml. The asterisks indicate a statistically significant difference ($p<0.05$) between subclass antibody ± Ab10523. Panels B, D, and F: reversal of human IgG subclass inhibition by the combination of blocking antibodies to CD32b and CD32a. Panel G; cells were stimulated with 14 nM NP-BSA (1 μg/ml) and the concentration of subclass IgG varied (n=3). (●) IgG1, (○) IgG2, (■) IgG3. Panel H; reversal of inhibition by the inclusion of Ab10523 at 50 μg/ml for each of the curves shown in panel G.