Published in final edited form as:

J Immunol Methods. 2012 September 28; 383(1-2): 60–64. doi:10.1016/j.jim.2012.05.016.

Assessing basophil functional measures during monoclonal anti-IgE therapy

Sarbjit S. Saini, MD and Donald W. MacGlashan Jr., MD, PhD

Abstract

Several decades of work support that measures of allergen responses of IgE-bearing peripheral blood basophils can reflect clinical expression of allergic disease. Basophils are recognized to respond to allergen exposure with a variety of pro-inflammatory mediators such as histamine, leukotrienes and cytokines such as IL-4 and IL-13. Furthermore, the suppression of established basophil allergen responses has been observed as a consequence of current treatments such as specific allergen immunotherapy (SIT) for allergic airways disease and most recently, in oral immunotherapy for food allergy. In the last decade, an immune-based therapy targeting IgE, omalizumab, has emerged as an adjunct treatment for a variety of allergic diseases. This monoclonal humanized IgG antibody specifically binds circulating IgE at a region in the Fc tail that prevents IgE attachment to high affinity IgE receptor (FcεRI) bearing cell types such as tissue mast cells and blood basophils. As a result of omalizumab capture of IgE, these cells have a significant reduction in surface-bound IgE, FcεRI receptor levels, and their capacity to respond to allergen exposure with mediator release. This review focuses on methods to monitor changes of basophil allergen reactivity with a focus on omalizumab therapy and the implications for clinical disease management.

Keywords

Basophils; Activation markers; Omalizumab

Introduction

Assessing the impact of therapeutic interventions on the clinical and immunologic responses of allergic subjects is a topic of extensive investigation. Available approaches include the measurement of *in vivo* allergen challenge responses (e.g., skin tests, bronchial challenge, nasal challenge, oral food challenge), serologic measures, or *in vitro* studies of cells that participate in the allergic reaction. Several decades of work support that measures of allergen responses of IgE-bearing peripheral blood basophils can reflect clinical expression of allergic disease[1, 2]. Basophils are recognized to respond to allergen exposure with a variety of pro-inflammatory mediators such as histamine, leukotrienes and cytokines such as IL-4 and IL-13. Furthermore, the suppression of established basophil allergen responses has been observed as a consequence of current treatments such as specific allergen immunotherapy (SIT) for allergic airways disease and most recently, in oral immunotherapy

© 2012 Elsevier B.V. All rights reserved.

Contact Information: Room 2B. 71B, 5501 Hopkins Bayview Circle, Baltimore, MD 21224, Telephone: 410-550-2129, Fax: 410-550-2527, ssaini@jhmi.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

for food allergy[3]. In the last decade, an immune-based therapy targeting IgE, omalizumab, has emerged as an adjunct treatment for a variety of allergic diseases[4]. This monoclonal humanized IgG antibody specifically binds circulating IgE at a region in the Fc tail that prevents IgE attachment to high affinity IgE receptor (FcεRI) bearing cell types such as tissue mast cells and blood basophils. As a result of omalizumab capture of IgE, these cells have a significant reduction in surface-bound IgE, FcεRI receptor levels, and their capacity to respond to allergen exposure with mediator release [5]. This review focuses on methods to monitor changes of basophil allergen reactivity with a focus on omalizumab therapy and the implications for clinical disease management.

Role of basophils in allergic disease

The historical view of basophils in allergic disease was built on the idea that this blood cell served as the circulating counterpart of tissue mast cells. Recognition of the unique capacity of basophils to secrete a range of mediators such as histamine and cytokines (IL-4, IL-13), migrate to sites of allergic inflammation, and promote Th-2 type inflammation has altered the perception of basophils as a major contributing cell in allergic disease [1]. Recent murine models have also suggested a role for basophils in antigen presentation, but this is controversial [6]. The evidence for a role of basophils in human allergic disease dates to the 1970's, when Lichtenstein and colleagues found that seasonal symptoms of ragweed related allergic rhinitis were correlated with the degree of basophil histamine release to ragweed *in vitro* [2]. More recently, a robust *in vitro* basophil cat allergen histamine response was found to have a strong predictive value for a positive clinical outcome to an experimental nasal allergen challenge to the same cat allergen [7]. In a majority of children with food allergy, basophils display enhanced spontaneous histamine release that appears partly related to ongoing food allergen exposure [8, 9]. Other studies have examined the relationship of basophil food-allergen reactivity to the clinical degree of food allergy. For example, a study comparing milk allergic children with a range of clinical reactivity (reactions to uncooked milk versus reactions to both cooked and uncooked milk protein) found that reduced *in vitro* basophil reactivity to milk allergen is associated with clinical tolerance to cooked milk protein ingestion [10]. These selected studies highlight a relationship between measures of *in vitro* basophil reactivity and *in vivo* allergen reactivity that form the rationale for monitoring basophil allergen reactivity to assist predictions of clinical reactivity.

Measures of basophil allergen responses in allergen immunotherapy

Many studies of specific allergen immunotherapy (SIT) routinely have utilized the blood basophil allergen driven histamine release as a readout of immunologic changes associated with therapy. Studies by Cook used passive transfer of pre and post ragweed SIT serum to non-allergic recipients to first demonstrate the presence of a specific blocking antibody in the post SIT serum that altered the skin test response [11]. It was also noted that allergen specific IgG rise correlates with falls in specific IgE during SIT [2]. In the 1980's, it was noted that basophil cell-bound IgE, RAST and cell sensitivity all correlated in grass allergic subjects, but in grass SIT treated patients these same parameters lacked correlation [12]. During early SIT, increased basophil cell-bound specific IgE occurred as a result of elevated allergen specific IgE, yet a reduction in basophil allergen sensitivity was seen in the same subjects. Collectively, these studies indicated that the basophil allergen response was being inhibited by a competing, allergen-specific IgG as a result of SIT. In general, SIT results in allergen specific alterations in the basophil mediator response while global cellular responses are thought to be preserved. The current view is that allergen specific IgG directly competes for allergen binding with basophils bearing allergen-specific IgE, and thereby reduces their mediator response. In order to observe this effect, however, serum from SIT-treated patients (either autologous or heterologous) must be included in the *in vitro* allergen

challenge so that the IgG antibodies that are present can block allergen binding to cell bound IgE. The generation of competing, allergen-specific IgG antibodies as a result of SIT is widely accepted and also attributed to part of the efficacy measures of this treatment [3, 13]. The link between SIT induced specific IgG antibody and impairment of basophil allergen responses is clear, and has been demonstrated in the course of immunotherapy for venoms[14], food [15]and pollens[16]. However, the linkage of such basophil changes to clinical reactivity remains less clear and is an area of active research [17]. However, an absence of change in basophil allergen reactivity has been associated with poor clinical outcomes in the setting of SIT (see below).

Newer studies have adopted an alternate metric of basophil allergen reactivity that involves flow cytometric measurement of surface markers that reflect cell activation. One commonly used marker is CD63, a membrane of the LAMP family of proteins that reside on the membranes of cytoplasmic granules containing histamine. When the basophils experience a stimulus such as allergen or of another receptor pathway (e.g. FMLP) that activates the histamine degranulation response, CD63 is generally exposed on the external cell surface through the fusion of granule with the plasma membrane[18]. While a direct correlation between measures of cellular histamine release and levels of induced CD63 surface marker expression might be expected[19], there are notable exceptions based on comparisons of a variety of stimuli generating histamine release [20-23]. Among the explanations for this discrepancy in correlations between CD63 and histamine release are the type of stimulus used (IgE receptor dependent or independent), the strength of the calcium signal induced, and the type of vesicle degranulation behavior induced[20]. In basophils, histamine vesicles can be released as smaller vesicle particles that merge with the plasma membrane in a process called piece-meal degranulation [24, 25]. In contrast, stimuli that generate more intense calcium signals can trigger the shift to an alternate histamine vesicle behavior, leading to the fusion of multiple vesicles to form larger degranulation sacs that eventually merge with the plasma membrane. This latter degranulation behavior, termed anaphylactic degranulation is more likely to be observed with marked elevation of CD63[22]. It is also notable that the dose response curves for stimuli yielding optimal histamine release may not overlap with that for optimal CD63 expression. For example, a supraoptimal dose of allergen can lead to reduced overall histamine release while the measured CD63 levels still remain elevated. Thus, the interpretation of the relationship of mediator release to surface activation marker profiles of basophils must be done with a full knowledge of the dose response behavior for each independent outcome[22].

A second surface activation marker commonly used is CD203c, which is limited in expression to basophils, mast cells and their progenitors[26]. This molecule is an ectonucleotide pyrophosphatase with as yet undefined function. This marker is rapidly elevated on the surface of the basophil with IgE receptor crosslinking or IL-3 exposure and can exist at low level on resting basophils[27, 28]. An obvious advantage relative to CD63 is the restricted expression of this marker on the cells of interest, but as noted above for CD63, elevation of this marker also does not always reflect the magnitude of mediator release such as histamine. Further, signaling pathways that regulate the upregulation of CD203c, CD 63 and histamine release are not fully understood but clearly have distinct profiles [20, 22, 23].

These flow dependent assays of allergen-induced basophil activation have been applied to monitor the efficacy of allergen SIT and also in studies with omalizumab [29, 30]. In some studies, this assay readout has been referred to as basophil CD sens which is the inverted value ($\times 100$) of the allergen concentration yielding 50% of the maximal marker elevated response achieved [31]. A modified birch pollen allergen was studied in SIT and led to the expected rise in allergen-specific IgG antibodies. Also, post-SIT serum as compared to pre-SIT serum inhibited the allergen-induced basophil expression of CD63 response, and this

effect was lost with IgG depletion of post SIT serum [32]. Along the same lines, treatment failure of SIT, such as venom SIT has been related to a lack of change in basophil reactivity to venom based on CD63 reactivity in a whole blood assay. However, no difference in IgE levels or IgG4 levels was seen among the treated subjects but the diversity and affinity of the specific IgG response was not measured [33]. Studies of the side effects to venom SIT have also used basophil sensitivity calculations in predicting serious events (SE) in VIT [34] and shown that high basophil sensitivity predicts SE's in VIT [35]. While the main mechanism proposed for basophil activity suppression via SIT appears to be the generation of competitive IgG antibodies, there remains the possibility of also involving basophil IgG receptors that could inhibit IgE receptor activation pathways. At present, the data to support such IgG receptor engagement is limited and conflicting [36, 37].

Omalizumab studies monitoring basophil outcomes

A recent detailed study of cat allergic subjects treated with omalizumab allowed the demonstration of the kinetics and magnitude of alterations in blood basophils allergen responses [38]. Among the expected outcomes was the rapid decline in free IgE that resulted in an overall 80% decline in basophil surface IgE and IgE receptors by 2 weeks of initiation of therapy, consistent with past observations [5, 39, 40]. However, the effect of omalizumab on the reduction of basophil allergen-induced mediator responses revealed a dependence on the ratio of allergen specific IgE relative to total serum IgE, also termed specific activity[41]. For example, an individual with a level of cat specific IgE that constitutes less than 3% of their total IgE was found to rapidly lose in vitro basophil histamine release to cat allergen entirely within the early weeks of omalizumab therapy. In contrast, individuals with cat allergen specific IgE ratios >4% were noted to demonstrate a loss of sensitivity for cat allergen mediated histamine response without a complete loss in their capacity for mediator response [38]. Thus, while all omalizumab recipients experienced reductions in cat allergen evoked basophil mediator response, there was marked heterogeneity in both the timing and degree of reduction. The clinical implications of full reduction in basophil allergen response relative to partial reduction remain to be elucidated in larger studies.

A separate series of studies to monitor changes in basophil allergen sensitivity under the influence of omalizumab involved flow cytometric measurements of allergen induced activation marker elevation. Nopp and colleagues studied timothy grass pollen allergic subjects for grass allergen -induced basophil CD63 elevation, end point skin test titration, and nasal provocation challenge responses [29]. A positive relationship was described between basophil CD sens and specific IgE levels as well as the lowest provocative dose for nasal allergen challenge symptoms. These indexed measures were first determined in a reference group of grass allergic subjects and next compared to CDsens measures in grass allergic subjects who had received 4 years of omalizumab. It was noted that omalizumab recipients had a lower CDsens to grass allergen than the untreated reference group of grass allergic subjects. Further, in a small group of subjects CDsens was noted to fall after the initiation of omalizumab treatment[29]. It should be noted that the CDsens method of evaluating the basophil response captures an important aspect of the allergen-mediated reaction that is missed if only a single concentration of allergen is used to assess the basophil response (usually a high concentration). Some of the pitfalls of too much simplification of the basophil assay have been reviewed elsewhere[42]. In this context, it is known that IL-3 alters a variety of signaling pathways in the basophil and shifts its behavior in patient-specific ways that may disguise some of the interesting underlying biology that occurs during clinical therapies. Thus, its use in a standard assay of basophil function should be done with caution and probably avoided.

Omalizumab induced reduction of serum IgE, blood basophils IgE receptors, and allergen induced mediator response are known to reverse after the discontinuation of therapy and return to pre-treatment values[43]. A separate study examined allergic subjects after 6 years dosing with omalizumab for CD sens measures relative to a reference group of untreated subjects with specific allergen sensitivity to cat or dust mite allergen. All the omalizumab recipients were considered to be clinical responders to therapy by several clinical outcome measures. During the first 12 months after therapy withdrawal CDsens rose suggesting that the basophils were regaining sensitivity (up to 20-fold after therapy), but still had lower CD sens values than the reference group of untreated allergic subjects[44]. This reduced basophil reactivity also appeared to be independent of changes in allergen specific IgG levels. A later assessment three years after stopping treatment showed a persistence of reduced CD sens relative to the reference group [45]. Survey measures of clinical disease control suggested that reduced CD sens was found in the subjects with persistent control of their asthma symptoms. Of note, allergen specific to total IgE ratios did not differ before, during and after the withdrawal of omalizumab.

Basophil outcomes relative to clinical measures in omalizumab recipients

At the lower range of total serum IgE in the allergic subject population, it is noted that specific activity IgE is often higher and thus basophil sensitivity is greater based on measured CD sens [46]. The relationship between specific activity levels, basophil CDsens and clinical measures of disease was therefore investigated in a 16-week double-blind, placebo-controlled clinical trial of omalizumab in 59 asthma subjects with cat allergy. Enrolled subjects were stratified such that 28 had cat IgE specific activity of <1% while the other 31 had cat IgE specific activity >3.8% [47]. As expected, basophil CD sens using CD63 was noted to be directly related to the specific activity ratio for cat allergen specific IgE. On therapy, CD sens was increased in both groups of actively treated subjects relative to pre-treatment. Remarkably, CD sens was completely lost in 13 of the 18 actively treated subjects with low cat specific activity (<1%), an effect similar to the loss of allergen induced basophil mediator response in the Eckman study[38]. Despite these marked basophil changes between the 2 groups, a clear clinical difference between the high and low specific activity treatments groups or between treatment and placebo was not demonstrated based on a simple disease survey.

Besides specific activity, several other factors can regulate basophil allergen sensitivity measures, such as the signaling molecule Syk. Levels of Syk are critical to initiate the downstream cascade towards mediator release after IgE receptor activation. An absence of Syk protein levels can lead to marked reduction in mediator release from activated basophils [48]. In contrast, it has also been recently noted that levels of Syk protein rise roughly 2-fold in subjects who received omalizumab treatment resulting in an increase in the mediator release to a pan-IgE crosslinking stimulus [39] and the changes in syk expression are well-correlated to the increased responsiveness to the pan-IgE crosslinking stimulus on a patient-by-patient basis. This suggests that IgE levels contribute to the regulation of Syk protein levels in blood basophils although how these two parameters would be related would be speculative [39]. There is the possibility that measurement of Syk levels in basophils, or the change in responsiveness to a pan-IgE crosslinking stimulus, along with other factors such as specific activity may be related to clinical outcomes.

In the Eckman study, a repeat cat nasal allergen challenge was conducted at the earliest time when basophil in vitro allergen reactivity was suppressed greater than 80% from baseline or after 45 days of treatment. Remarkably, the early phase clinical symptoms and sneezes were reduced 50 % reduction at a time when blood basophil in vitro cat allergen responses were reduced but nasal and skin mast cell allergen response measures were similar to pre-

omalizumab therapy measures [38]. Thus, reduction in basophil allergen reactivity in this second study revealed a role for the basophil to either participate in the nasal allergic reaction or simply reflect a change in the host nasal organ allergic response.

A third recent study involving peanut allergic subjects noted that basophils at baseline expressed elevated CD203c and further elevated CD203c expression with in vitro peanut allergen exposure. In five subjects treated with omalizumab, basal and in vitro allergen stimulated CD203c levels were reduced and noted to rebound off therapy[49]. Examination of basophil CD63 responses under the same conditions as basophil CD203c proved to be less reliable index in the nut allergic subjects.

Unpublished studies derived from the omalizumab study by Eckman et al. [38] examined the relationship between allergen-induced up-regulation of CD203c expression on basophils and histamine release from the same samples. In the most general sense, the CD203c biomarker demonstrated changes in basophil function that were commensurate with the changes observed in histamine release. However, it was also apparent that in detail, on a patient-by-patient basis, the kinetics of the changes and the magnitudes of the changes were not particularly concordant. Since recent studies have begun to demonstrate that there are different signaling pathways by which CD203c expression and histamine release occur, it is not very surprising that discordance occurs during rapid changes in the clinical status of the patient [23].

Summary

Several decades of work support the usefulness of basophil allergen reactivity measures in the expression of clinical disease and during traditional allergen immunotherapy. Newer basophil surface activation markers can indicate basophil allergen sensitivity but are not surrogates for allergen induced mediator responses such as histamine release. The success of newer monoclonal anti-IgE therapy in several allergic diseases has prompted an examination of basophil allergen reactivity shifts along with limited studies of clinical reactivity measures. Further studies with omalizumab are needed to evaluate the potential for predicting clinical outcomes based on the measures of basophil allergen response.

References

1. Schroeder JT. Basophils beyond effector cells of allergic inflammation. *Adv Immunol.* 2009; 101:123–61. [PubMed: 19231594]
2. Lichtenstein LM, et al. IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. *J. Clin. Invest.* 1973; 52:472–482. [PubMed: 4119162]
3. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol.* 2011; 127(1):18–27. quiz 28–9. [PubMed: 21211639]
4. Kopp MV. Omalizumab: Anti-IgE therapy in allergy. *Curr Allergy Asthma Rep.* 2011; 11(2):101–6. [PubMed: 21243454]
5. Beck L, et al. Omalizumab induced reductions in mast cell FcεRI expression and function. *J Allergy Clin Immunol.* 2004; 114:527–30. [PubMed: 15356552]
6. Schroeder JT. Basophils: emerging roles in the pathogenesis of allergic disease. *Immunol Rev.* 2011; 242(1):144–60. [PubMed: 21682743]
7. Paterniti M, et al. Cat allergen-induced blood basophil reactivity in vitro predicts acute human nasal allergen challenge responses in vivo. *Clin Exp Allergy.* 41(7):963–9. [PubMed: 21668817]
8. May CD. High spontaneous release of histamine in vitro from leukocytes of persons hypersensitive to food. *J Allergy Clin Immunol.* 1976; 58(3):432–7. [PubMed: 61221]
9. Sampson HA, Broadbent KR, Bernhisel-Broadbent J. Spontaneous release of histamine from basophils and histamine-releasing factor in patients with atopic dermatitis and food hypersensitivity. *N. Engl. J. Med.* 1989; 321:228–232. [PubMed: 2473400]

10. Wanich N, et al. Allergen-specific basophil suppression associated with clinical tolerance in patients with milk allergy. *J Allergy Clin Immunol.* 2009; 123(4):789–94. e20. [PubMed: 19348919]
11. Cooke RA, et al. Serological Evidence of Immunity with Coexisting Sensitization in a Type of Human Allergy (Hay Fever). *J Exp Med.* 1935; 62(6):733–50. [PubMed: 19870445]
12. Malling HJ, et al. Basophil histamine release and humoral changes during immunotherapy. Dissociation between basophil-bound specific IgE, serum value, and cell sensitivity. *Allergy.* 1982; 37(3):187–90. [PubMed: 6182814]
13. Lalek N, et al. Immunoglobulin G-dependent changes in basophil allergen threshold sensitivity during birch pollen immunotherapy. *Clin Exp Allergy.* 2010; 40(8):1186–93. [PubMed: 20528883]
14. Ebo DG, et al. The basophil activation test in the diagnosis and follow-up of hymenoptera venom allergy: an alternative point of view. *J Invest Allergol Clin Immunol.* 2008; 18(6):493–4.
15. Jones SM, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol.* 2009; 124(2):292–300. 300 e1-97. [PubMed: 19577283]
16. Kawakami A, et al. Blocking antibody is generated in allergic rhinitis patients during specific immunotherapy using standardized Japanese cedar pollen extract. *Int Arch Allergy Immunol.* 2008; 146(Suppl 1):54–60. [PubMed: 18504408]
17. Van Overtvelt L, et al. Changes in basophil activation during grass-pollen sublingual immunotherapy do not correlate with clinical efficacy. *Allergy.* 2011; 66(12):1530–7. [PubMed: 21883279]
18. Ebo DG, et al. In vitro allergy diagnosis: should we follow the flow? *Clin Exp Allergy.* 2004; 34(3):332–9. [PubMed: 15005724]
19. Knol EF, et al. Monitoring human basophil activation via CD63 monoclonal antibody-435. *J. Allergy Clin. Immunol.* 1991; 88(3):328–338. [PubMed: 1716273]
20. Hennesdorf F, et al. Identification of CD13, CD107a, and CD164 as novel basophil-activation markers and dissection of two response patterns in time kinetics of IgE-dependent upregulation. *Cell Res.* 2005; 15(5):325–35. [PubMed: 15916720]
21. MacGlashan DW Jr. Graded changes in the response of individual human basophils to stimulation: distributional behavior of events temporally coincident with degranulation. *J Leukoc Biol.* 1995; 58(2):177–88. [PubMed: 7543919]
22. MacGlashan D Jr. Expression of CD203c and CD63 in human basophils: relationship to differential regulation of piecemeal and anaphylactic degranulation processes. *Clin Exp Allergy.* 2010; 40(9):1365–77. [PubMed: 20633031]
23. MacGlashan D Jr. Marked differences in the signaling requirements for expression of Cd203c and CD11b versus CD63 expression and histamine release in human basophils. *Int Arch Allergy Immunol.* 2011 in press.
24. Dvorak AM, et al. An ultrastructural analysis of tumor-promoting phorbol diester-induced degranulation of human basophils. *Am. J. Pathol.* 1992; 141(6):1309–1322. [PubMed: 1466396]
25. Dvorak AM, et al. F-met peptide-induced degranulation of human basophils. *Lab. Invest.* 1991; 64(2):234–253. [PubMed: 1705302]
26. Buhring HJ, Streble A, Valent P. The basophil-specific ectoenzyme E-NPP3 (CD203c) as a marker for cell activation and allergy diagnosis. *Int Arch Allergy Immunol.* 2004; 133(4):317–29. [PubMed: 15031605]
27. Hauswirth AW, et al. Interleukin-3 promotes the expression of E-NPP3/CD203C on human blood basophils in healthy subjects and in patients with birch pollen allergy. *Int J Immunopathol Pharmacol.* 2007; 20(2):267–78. [PubMed: 17624239]
28. Vasagar K, et al. Evidence of In Vivo Basophil Activation in Chronic Idiopathic Urticaria. *Clin Exp Allergy.* 2006; 36(6):770–6. [PubMed: 16776678]
29. Nopp A, et al. Basophil allergen threshold sensitivity: a useful approach to anti-IgE treatment efficacy evaluation. *Allergy.* 2006; 61(3):298–302. [PubMed: 16436137]
30. Nopp A, et al. CD-sens: a biological measure of immunological changes stimulated by ASIT. *Allergy.* 2009; 64(5):811–4. [PubMed: 19220221]

31. Johansson SG, et al. Passive IgE-sensitization by blood transfusion. *Allergy*. 2005; 60(9):1192–9. [PubMed: 16076307]
32. Ceuppens JL, et al. Immunotherapy with a modified birch pollen extract in allergic rhinoconjunctivitis: clinical and immunological effects. *Clin Exp Allergy*. 2009; 39(12):1903–9. [PubMed: 20085600]
33. Peternelj A, et al. Basophil sensitivity in patients not responding to venom immunotherapy. *Int Arch Allergy Immunol*. 2008; 146(3):248–54. [PubMed: 18270492]
34. Eberlein-Konig B, et al. Predicting side-effects in venom immunotherapy by basophil activation? *Allergy*. 2006; 61(7):897. [PubMed: 16792593]
35. Kosnik M, et al. High sensitivity of basophils predicts side-effects in venom immunotherapy. *Allergy*. 2005; 60(11):1401–6. [PubMed: 16197472]
36. Cady CT, et al. IgG antibodies produced during subcutaneous allergen immunotherapy mediate inhibition of basophil activation via a mechanism involving both FcγRIIA and FcγRIIB. *Immunol Lett*. 2010; 130(1-2):57–65. [PubMed: 20004689]
37. Ejmaes AM, et al. Inhibition of rBet v 1-induced basophil histamine release with specific immunotherapy -induced serum immunoglobulin G: no evidence that FcγRIIB signalling is important. *Clin Exp Allergy*. 2006; 36(3):273–82. [PubMed: 16499637]
38. Eckman JA, et al. Effects of omalizumab on basophil and mast cell responses using an intranasal cat allergen challenge. *J Allergy Clin Immunol*. 2010; 125(4):889–895. [PubMed: 19962744]
39. Zaidi AK, Saini SS, Macglashan DW Jr. Regulation of Syk kinase and FcRβ expression in human basophils during treatment with omalizumab. *J Allergy Clin Immunol*. 2010; 125(4):902–908. e7. [PubMed: 20236696]
40. MacGlashan DW Jr. et al. Down-regulation of FcεRI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol*. 1997; 158(3):1438–45. [PubMed: 9013989]
41. Hamilton RG, MacGlashan DW Jr. Saini SS. IgE antibody-specific activity in human allergic disease. *Immunol Res*. 2010; 47(1-3):273–84. [PubMed: 20066506]
42. Kleine-Tebbe J, et al. Diagnostic tests based on human basophils: potentials, pitfalls and perspectives. *Int Arch Allergy Immunol*. 2006; 141(1):79–90. [PubMed: 16837789]
43. Saini SS, et al. Down-regulation of human basophil IgE and FcεRIα surface densities and mediator release by anti-IgE-infusions is reversible in vitro and in vivo. *J Immunol*. 1999; 162(9):5624–30. [PubMed: 10228046]
44. Nopp A, et al. CD-sens and clinical changes during withdrawal of Xolair after 6 years of treatment. *Allergy*. 2007; 62(10):1175–81. [PubMed: 17845588]
45. Nopp A, et al. After 6 years with Xolair; a 3-year withdrawal follow-up. *Allergy*. 2010; 65(1):56–60. [PubMed: 19796193]
46. Johansson SG, et al. The importance of IgE antibody levels in anti-IgE treatment. *Allergy*. 2006; 61(10):1216–9. [PubMed: 16942572]
47. Johansson SG, et al. The size of the disease relevant IgE antibody fraction in relation to 'total-IgE' predicts the efficacy of anti-IgE (Xolair) treatment. *Allergy*. 2009; 64(10):1472–7. [PubMed: 19393000]
48. Lavens-Phillip S, MacGlashan D. The tyrosine kinases, p53 lyn, p72 syk are differentially expressed at the protein level, not the mRNA level in non-releasing human basophils. *Am J Cell and Respir Mole Med*. 2000; 23:566–571.
49. Gernez Y, et al. Basophil CD203c levels are increased at baseline and can be used to monitor omalizumab treatment in subjects with nut allergy. *Int Arch Allergy Immunol*. 2011; 154(4):318–27. [PubMed: 20975283]