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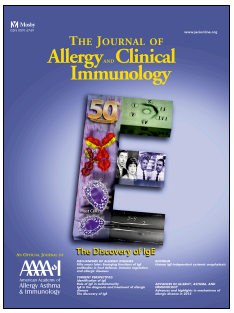

Basophil FcεRI expression is linked to time to omalizumab response in chronic spontaneous urticaria

Gustavo Deza, MD, Marta Bertolín-Colilla, MD, Silvia Sánchez, BSN, Dulce Soto, BSN, Ramon M. Pujol, MD, PhD, Ramon Gimeno, MD, PhD, Ana M. Giménez-Arnau, MD, PhD

PII: S0091-6749(18)30313-0

DOI: 10.1016/j.jaci.2018.02.021

Reference: YMAI 13329

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 26 September 2017

Revised Date: 8 January 2018

Accepted Date: 5 February 2018

Please cite this article as: Deza G, Bertolín-Colilla M, Sánchez S, Soto D, Pujol RM, Gimeno R, Giménez-Arnau AM, Basophil FcεRI expression is linked to time to omalizumab response in chronic spontaneous urticaria, *Journal of Allergy and Clinical Immunology* (2018), doi: 10.1016/j.jaci.2018.02.021.

Basophil FcεRI expression is linked to time to omalizumab response in chronic spontaneous urticaria

Authors: Gustavo Deza, MD[1], Marta Bertolín-Colilla, MD[1], Silvia Sánchez, BSN[1], Dulce Soto, BSN[2], Ramon M. Pujol, MD, PhD[1], Ramon Gimeno, MD, PhD[2 *], Ana M. Giménez-Arnau, MD, PhD[1 *]

Affiliations:

(1) Department of Dermatology, Hospital del Mar- Institut Mar d'Investigacions Mèdiques, Universitat Autònoma de Barcelona (UAB). Barcelona, Spain.
(2) Department of Immunology, Hospital del Mar- Institut Mar d'Investigacions Mèdiques. Barcelona, Spain.

*These authors contributed equally to this work.

Corresponding author:
Ana M. Giménez-Arnau, M.D., PhD
Department of Dermatology
Hospital del Mar- Institut Mar d'Investigacions Mèdiques, Universitat Autònoma de Barcelona (UAB)
Passeig Marítim, 25-29, 08003 Barcelona, Spain
Telephone: (+34) 932483380.
Fax: (+34) 932483328
E-mail: anamariagimenezarnau@gmail.com, 22505aga@comb.cat

Conflict of interests: Ana M. Giménez-Arnau is a medical Advisor for Uriach Pharma,

Genentech and Novartis. She has received research grants from Intendis-Bayer, Uriach Pharma and Novartis, and has participated in educational activities sponsored by Uriach Pharma, Novartis, Genentech, Menarini, Glaxo Smith & Kline, Merck MSD, Almirall and Leo Pharma. The rest of the authors declare that they have no relevant conflicts of interest.

Funding Sources: None

References: 9
Tables: 1
Figures: 1

Short Summary: This study suggests that baseline levels of basophil FcεRI receptor may predict time to response to anti-IgE therapy in chronic spontaneous urticaria.

Keywords: Basophil, FcεRI receptor, omalizumab, response, urticaria.

Abbreviations: ASST, autologous serum skin test; BHRA, basophil histamine release assay; CSU, chronic spontaneous urticaria; FcεRI, high-affinity IgE receptor; FR, fast responders; MFI, mean fluorescence intensity; SR, slow responders.

To the Editor:

Omalizumab has demonstrated excellent efficacy for the treatment of chronic spontaneous urticaria (CSU) in several randomized clinical trials and real-world studies.[1,2] As a monoclonal anti-IgE antibody, omalizumab prevents IgE binding to the high-affinity IgE receptor (FcεRI) on the surface of basophils and mast cells, resulting in a significant reduction in FcεRI levels on these cells and, therefore, in their capacity to respond to allergen exposure.[3] In this regard, the potential for predicting clinical outcomes during anti-IgE therapy based on basophil allergen response have been investigated in several studies mainly focused on allergic respiratory diseases.[4] In CSU, meanwhile, it has been recently demonstrated that the modulation of the basophil FcεRI expression plays a key role in the clinical improvement observed during omalizumab therapy.[3,5] Thus, a significant drop in the basophil FcεRI expression is immediately observed after the first dose that is maintained throughout the duration of the treatment.[5] Although this phenomenon seems to occur very soon after starting treatment, timing to satisfactory clinical response to omalizumab can be highly variable. The purpose of this study was to evaluate the evolution of the basophil FcεRI expression in patients with CSU showing a fast and slow clinical response to anti-IgE therapy, with the goal of a better characterization of such individuals and to examine potential associations for these response patterns.

This prospective study included patients with active CSU whose symptoms were not controlled with H1-antihistamines (even up to 4 times the recommended dose). These subjects were treated with omalizumab 300mg every 4 weeks, and peripheral blood samples were obtained prior to the 1st (baseline), 2nd, 3rd and 6th injection. Basophil FcεRI expression was measured by flow cytometry from these samples following standard procedures (Online Repository), and the levels were expressed as mean fluorescence intensity (MFI). Response to treatment was defined as an improvement of the patients' signs and symptoms achieving a weekly Urticaria Activity Score (UAS7) $\leq$6.[2,6] The cut-off time chosen for distinguishing fast responders (FR) and slow responders (SR) was 4 weeks, which corresponds to the usual first visit after starting treatment. To avoid potential interferences, patients who received anti-IgE therapy before, or were diagnosed of a chronic pruritic disease other than CSU (e.g. atopic dermatitis) were excluded from the study. It should be also noted that, since FcεRI expression in non-responders to omalizumab has been extensively studied in a recent

paper,[5] these subjects were excluded from the present analysis. The local clinical research ethics committee granted ethical approval for the study (2012/4913/I).

A total of 44 patients (31 women) responded to treatment with omalizumab. Thirty-three (75%) out of 44 patients responded within 4 weeks and were classified as FR, whereas the remaining 11 (25%) required more administrations to respond (SR; median time to response: 20 weeks; range, 8-36). No significant differences regarding clinical and demographic features were found between both groups (Table I). For clinical reasons, the autologous serum skin test (ASST) was performed only in 37 patients, being positive in 50% (14/28) and 77.8% (7/9) of the FR and SR, respectively (p=0.248; Fisher exact test). Regarding the basophil FcεRI expression, the median value at baseline was significantly higher in the FR (median 13247 of MFI; range 6753-25281) than in the SR (median 8428 of MFI; range 5720-17375) (p=0.002; Mann-Whitney U test; Fig.1A). However, no significant differences were observed regarding the evolution of the FcεRI expression during anti-IgE therapy between FR and SR, showing both a sharp reduction at 4 weeks that was maintained throughout the duration of the treatment (p>0.05 at 4,8 and 20 weeks; Test for the equality of medians; Fig.1B). Finally, a positive correlation was found between levels of total serum IgE and baseline FcεRI expression (Pearson's correlation coefficient r=0.355; p=0.018; Fig.1C).

Few data are available regarding the patterns of response to omalizumab in CSU. Data from phase III clinical trials suggest that there might be two categories of responders: those who respond after just one dose of omalizumab (within four weeks), and those who required two or more doses to achieve the disease control.[6] However, the exact mechanisms responsible for these responses remain unknown and, more importantly, immunological markers predicting this phenomenon are currently scarce. In this sense, Gericke *et al* postulated that serum autoreactivity may be associated with the time to symptom relief with anti-IgE therapy.[7] Thus, the authors found that a slow response could probably be predicted by a positive basophil histamine release assay (BHRA) and ASST. In other words, a slow response to omalizumab might occur mainly in patients with autoantibodies directed against either the cell-bound IgE or FcεRI. Although no significant differences were found between ASST and time to response in our population, a positive ASST result was more frequently observed in SR. Moreover, according with our analysis, baseline FcεRI expression may also be associated with the

time to response to omalizumab. Thus, patients with very high baseline levels might be more likely to achieve an early therapeutic response. A possible explanation is that these autoantibodies against FcεRI, which are presumably present in SR, could interfere in the measurement of basophil FcεRI expression by flow cytometry, causing lower levels than in FR. Apart from this difference, the FcεRI expression seems to exhibit a similar evolution during anti-IgE therapy in both categories of responders, showing a sharp drop immediately after the initial dose that is maintained during the subsequent administrations. This finding suggest that the FcεRI down-regulation may not be a definitive mechanism of action in some patients and therefore, the combination of more than one pharmacological mechanism seems necessary to fully explain the pattern of symptoms improvement seen with omalizumab therapy in CSU.

Regarding total IgE levels, previous studies have demonstrated a positive correlation between basophil FcεRI expression and total serum IgE levels in a variety of disease states, such as asthma, atopic dermatitis and hyper-IgE syndrome.[8] Similarly, this association could also be observed in patients with CSU. However, in this condition, which is not considered a classic allergic disease, the correlation seems to be weaker, suggesting that other factors may also have a significant influence on FcεRI expression. In addition, it has been recently postulated that total IgE levels could have an important role in predicting the clinical response to omalizumab in CSU.[9] In our study, although not significant on the 5% level, a trend towards lower IgE levels in SR was also noted. The value of total IgE as a useful biomarker of time to omalizumab response in CSU may be somewhat controversial, given the wide range and overlapping of values (therefore, not a clear distinction) observed in both categories of responders.

In summary, although further investigations are needed to confirm these preliminary observations, our study suggests the existence of a link between baseline levels of basophil FcεRI expression and time to response to omalizumab in CSU, with higher levels shown in FR. The knowledge of the patterns of therapeutic response would have an important impact in clinical practice, helping physicians to improve their approach on the patients' follow-up.

Gustavo Deza, MD[a]

Marta Bertolín-Colilla, MD[a]

Silvia Sánchez, BSN[a]

Dulce Soto, BSN[b]

Ramon M. Pujol, MD, PhD[a]

Ramon Gimeno, MD, PhD[b *]

Ana M. Giménez-Arnau, MD, PhD[a *]

From [a] *the Department of Dermatology and* [b] *the Department of Immunology, Hospital del Mar- Institut Mar d'Investigacions Mèdiques, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain.*

** These authors contributed equally to this work.*

ACKNOWLEDGMENTS

The authors would like to thank Xavier Duran (Mar Institute of Medical Research Foundation) for his help in conducting the statistical analysis.

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Table I. Baseline characteristics

	All responders (n=44) n (%) or median (range)	**Fast responders (n=33)** n (%) or median (range)	**Slow responders (n=11)** n (%) or median (range)	***p****
Age, *years*	49 (14-77)	49 (39-77)	44 (14-77)	0.322
Female	30 (68.2)	21 (63.6)	9 (81.8)	0.457
Disease duration (*months)*	60 (14-360)	60 (14-360)	84 (14-240)	0.842
UAS7 at baseline	25 (14-40)	24 (14-40)	26 (21-39)	0.285
Blood basophil count ($x10^3/\mu L$)	0.03 (0.01-0.11)	0.03 (0.01-0.09)	0.04 (0.01-0.11)	0.271
Total IgE levels at baseline (*kU/L)*	151 (17-1536)	200 (17-1536)	66 (34-889)	0.067
Positive ASST [a]	21 (56.8)	14 (50.0)	7 (77.8)	0.248
Previous treatment with cyclosporine	18 (40.9)	13 (39.4)	5 (45.5)	0.738

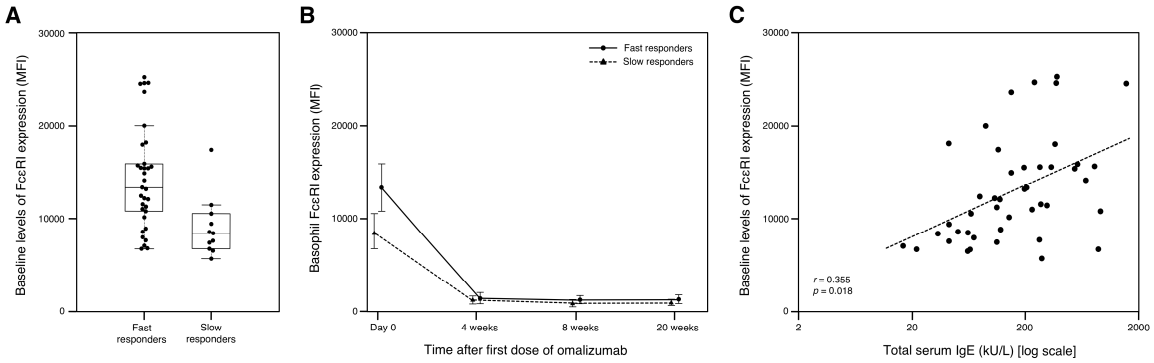
UAS7, weekly urticaria activity score; IgE, immunoglobulin E; ASST, autologous serum skin test.

[a] ASST was only assessed in 37 patients (28 fast responders and 9 slow responders).

* Statistical differences between fast and slow responders were analyzed using Fisher exact test or Mann-Whitney *U* test when appropriate.

FIGURE LEGENDS

Figure 1. Basophil FcεRI levels (expressed in mean fluorescence intensity; MFI) in CSU patients showing a satisfactory clinical response to omalizumab. **A,** Box-whiskers plots presenting median, interquartile range, maximum and minimum as well as individual dots of baseline FcεRI expression in fast and slow responders (p= 0.002; Mann-Whitney U test). **B**, Evolution of the basophil FcεRI expression (median, interquartile range) in both categories of responders during anti-IgE therapy. **C**, Correlation between baseline FcεRI expression and baseline total IgE levels (represented in linear and logarithmic [log] scale, respectively) in the 44 CSU patients included in the study (Pearson's correlation coefficient r= 0.355; p= 0.018).



ONLINE REPOSITORY MATERIAL

Methods

Basophil cell preparation and flow cytometry

Flow cytometry analysis was performed following standard procedures. Briefly, 150 µl anticoagulated blood was incubated on the same day of collection during 20 min at 4ºC with an excess of human immunoglobulins to block unspecific binding. Afterwards, blood was stained with either anti-CD123-PE (BD) or anti-CD193-APC (Miltenyi) to identify basophils and with anti-FcεR1a-FITC (clone CRA1, Ebiosciences) or an isotype control to establish the expression of FcεRI on the surface of blood basophils. The samples were then lysed and fixed using the FACS Lysing Solution (BD Biosciences) and analyzed by flow cytometry in a FACSCanto using the FACSDiva software. At least 2 x 10^5 events were acquired. Instrument settings (e.g. scatter and voltage settings and compensation matrix) and experimental conditions (e.g. antibody clones and dilution) remained strictly constant for all samples throughout the study. To ensure consistency in the analysis, the same investigator processed and analyzed all samples and two independent researchers correlated the levels of basophil FcεRI expression and the clinical scores.

Autologous serum skin test

The autologous serum skin test (ASST) was performed as previously described.[E1] Briefly, venous blood was taken before the first administration of omalizumab. Samples were centrifuged at 2500 rpm for 10 minutes and the serum separated. Afterwards, 50 mL of fresh undiluted autologous serum was injected intradermally into the patient's volar forearm. Similar volumes of 0.9% NaCl saline and 100 mg/mL histamine were used as negative and positive controls, respectively. A positive ASST was considered when the diameter of serum-induced wheal was >1.5mm compared to the saline-induced response at 30 minutes.

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