Relevance of the basophil high-affinity IgE receptor in chronic urticaria:

Clinical experience from a tertiary care institution

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ABBREVIATIONS:

- Anti-Tg - Anti-thyroglobulin
- Anti-TPO - Anti-thyroid peroxidase
- ASST - Autologous serum skin test
- APST - Autologous plasma skin test
- ATA - Anti-thyroid antibody
- CIndU - Chronic inducible urticaria
- CsTT - Critical stimulation time threshold
- CSU - Chronic spontaneous urticaria
- CTT - Critical temperature threshold
- CU - Chronic urticaria
- FcεRI - High-affinity IgE receptor
- HC - Healthy control
- Ig - Immunoglobulin
- MFI - Mean fluorescence intensity
- UAS7 - 7-day urticaria activity score
- UCT - Urticaria Control Test
ABSTRACT

Background. The high-affinity IgE receptor (FcεRI) expression on effector cells has been poorly characterized in patients with chronic urticaria (CU) to date.

Objectives. To investigate the FcεRI expression on blood basophils in a large cohort of CU patients and its potential relationship with relevant features of the disease.

Methods. Basophil FcεRI expression was measured by flow cytometry in 287 CU patients (192 with Chronic Spontaneous Urticaria and 95 with Chronic Inducible Urticaria) at their initial evaluation in our Department. A control group of healthy non-atopic individuals was included to provide reference data, and the effect of antihistamine and anti-IgE therapy on the basophil FcεRI expression was also evaluated in a cohort of CU patients.

Results. The median FcεRI expression was found significantly higher in CU patients compared to healthy controls (p<0.0001). A positive correlation was found between serum IgE levels and basophil FcεRI expression (R=0.422; p<0.001). Significantly higher FcεRI levels on basophils were detected in CU patients who presented with concomitant atopic features (p=0.003), negative autologous serum skin test (p=0.002), negative autologous plasma skin test (p=0.009) or undetected levels of anti-thyroid antibodies (p=0.01). Baseline FcεRI expression was not related with the activity and duration of the disease, and was not significantly modified during antihistamine therapy; however, it correlated with the clinical response to omalizumab (p=0.003).

Conclusion. Although further multicenter studies are needed to corroborate these findings, the assessment of basophil FcεRI levels might be relevant in daily clinical practice supporting an autoimmune pathogenesis and predicting response to anti-IgE treatment.
HIGHLIGHTS BOX

1. What is already known about this topic?
The activation of the high-affinity IgE receptor –FcεRI- on basophils and mast cells is crucial for the immediate hypersensitivity responses in subjects with atopic dermatitis, allergic asthma and allergic rhinitis.

2. What does this article add to our knowledge?
Basophil FcεRI expression is significantly upregulated in Chronic Spontaneous and Inducible Urticaria. Patients who present negative autologous serum/plasma skin test, undetected levels of anti-thyroid antibodies or satisfactory clinical response to omalizumab exhibit higher FcεRI levels.

3. How does this study impact current management guidelines?
Although further multicenter studies are needed to corroborate these findings, the assessment of basophil FcεRI expression might be relevant in daily clinical practice supporting an autoimmune pathogenesis and predicting response to anti-IgE treatment.

Keywords: Basophil, chronic urticaria, FcεRI, FcεRI expression, IgE receptor, omalizumab
INTRODUCTION

Chronic urticaria (CU) is a common skin condition characterized by the recurrent appearance of itchy wheals and/or angioedema for longer than 6 weeks.\(^1\) It is classified into two subtypes: chronic spontaneous urticaria (CSU), when the lesions occur without an obvious stimulus, and chronic inducible urticaria (CIndU), when symptoms are induced by different triggers, e.g. low temperatures, heat, pressure or exercise.\(^1,2\) Existing evidence demonstrates that CU symptoms may have major detrimental effects on quality of life, including daily activities and emotional well-being.\(^3\)

The pathophysiology of CU involves the activation and degranulation of effector cells, such as basophils and mast cells, and the subsequent release of pro-inflammatory/pathological mediators that play a key role in the development of CU symptoms.\(^4,5\) As of yet, it is unclear completely what causes such activation and degranulation. One of the most attractive explanation in most of the patients is the autoimmune mechanism, in which effector cells are activated by immunoglobulin (Ig) E or IgG through the high-affinity IgE receptor, FceRI, located on the surface of basophils, mast cells and antigen-presenting cells.\(^6-9\) Thereby, crosslinking of FceRI with the complex IgE-autoantigen (Type I autoimmunity) or with just IgG or the complex IgG-IgE (Type IIb autoimmunity) would cause the activation/degranulation of effector cells with the consequent release of preformed mediators and newly synthetized active substances.\(^6,10\)

Despite its supposed importance in the disease pathogenesis, FceRI expression on effector cells has been poorly characterized in CU patients to date. Therefore, we sought to investigate the FceRI expression on blood basophils in a large cohort of patients with CSU and CIndU to answer the following questions: (i) Are basophil FceRI levels increased in CU patients? (ii) Are there clinical features that modulate the FceRI
expression in patients with CSU or CIndU? (iii) Is FcεRI expression modified during treatment in CU patients? and more important, (iv) is the assessment of FcεRI expression relevant in daily clinical practice?

PATIENTS & METHODS

Subjects and study design

This prospective study included patients with CSU or CIndU referred to the Urticaria Clinic of the Department of Dermatology of Hospital del Mar (Barcelona) during the period from January 2014 to June 2018 (Ethical approval no. 2012/4913/I). Following a systematized clinical protocol, a thorough and structured history (including age, sex, disease duration, disease severity, personal history of atopic features [i.e. atopic dermatitis, allergic rhinitis and/or allergic asthma], presence of angioedema and concomitant subtypes of CU) and laboratory analyses (including total serum IgE levels, thyroid function and levels of anti-thyroid antibodies [ATAs]: anti-thyroid peroxidase [anti-TPO] and anti-thyroglobulin [anti-Tg]) were performed in all patients at the initial evaluation. Additionally, autologous serum skin test (ASST), autologous plasma skin test (APST) and/or standardized inducible testing were performed (when appropriate) as part of the routine study protocol. CIndU diagnosis was based on the patients’ clinical history and the results of standardized provocation testing.2 As the main objective of the present investigation, peripheral blood samples were obtained from CU patients to measure the FcεRI expression on basophils by flow cytometry. To avoid potential interferences, patients who were under treatment with biologic therapies (including omalizumab), oral corticosteroids and/or other immunosuppressive agents were excluded from the study. Blood samples from a group of healthy controls [HCs] without family and personal history of CU or atopic features were also evaluated to obtain reference data.
In addition, FceRI levels were assessed in a cohort of CU patients at different time points to investigate the effect of antihistamine and anti-IgE therapy on the basophil FceRI expression. For antihistamine treatment, FceRI levels were measured at the baseline evaluation and at least 1 month after the initiation of therapy (non-sedating H1-antihistamines, doses ranging from 1 to 4 times the recommended dose depending on the patient’s symptoms severity). Response to therapy was defined as an improvement in the patients’ signs and symptoms achieving a 7-day Urticaria Activity Score (UAS7, a composite score of itch severity and hive count over 7 days; range 0–42) ≤6 and/or an Urticaria Control Test (UCT, a validated tool for assessing disease control in daily practice; range 0–16) ≥12.1,11 On the other hand, as the effect of omalizumab on the FceRI expression in CSU patients has been extensively studied in recent investigations,12,13 we have focused on the analysis of the kinetic of FceRI levels during anti-IgE therapy in patients diagnosed with pure CIndU. Thus, FceRI expression was evaluated in CIndU patients who showed unsatisfactory response to antihistamines and were therefore treated with subcutaneous injections of omalizumab 300mg monthly. Basophil FceRI levels were measured on day 0 and on weeks 4, 8 and 20 of treatment (i.e. prior to the 1st, 2nd, 3rd and 6th injections); and response to therapy was evaluated at 6 months of treatment according to the UCT score.

Most primary endpoints of the study (e.g. evaluate the basophil FceRI expression as a potential biomarker of disease activity, disease duration, therapeutic response and for confirming CSU and distinguishing it from HCs) were pre-specified at the initiation of the investigation. Nevertheless, some observations, such as the FceRI-IgE correlation and the differences in FceRI expression regarding the “autoimmune” condition in CSU patients, were evaluated after the data collection was completed.
Basophil cell preparation and flow cytometry for FcεRI expression

Flow cytometry analysis was performed following standard procedures. Briefly, 150 μl of anticoagulated blood was incubated on the same day of collection during 20 min at 4°C with an excess of human immunoglobulins to block unspecific binding. Afterwards, blood was stained with anti-CD123-PE (BD Biosciences, San Jose, California) and anti-CD193-APC (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) to identify basophils and with anti-FcεR1a-FITC (clone CRA1, eBioscience, San Diego, California) or an isotype control to establish the expression of FcεRI on the surface of blood basophils. It should be taking into account that, after fine tuning the technique and evaluating the FcεRI expression using separately anti-CD123-PE and anti-CD193-APC markers, similar basophil FcεRI levels were observed in terms of mean fluorescence intensity (MFI; Figure S1); however, both antibodies were simultaneously used to avoid FcεRI positive dendritic cells. 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. Levels of the basophil FcεRI receptor were expressed as MFI.

Instrument settings (e.g. scatter and voltage settings and compensation matrix) and experimental conditions (e.g. antibody clones and dilution) remained 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.

Serum IgE levels, levels of ATAs and ASST/APST
Total IgE and levels of circulating anti-TPO and anti-Tg antibodies were analyzed in serum by chemiluminescence immunoassay technique using the IMMULITE 2000 XPi System (Siemens, Munich, Germany). The ASST was performed in CSU patients as previously described. Briefly, venous blood was taken at the initial evaluation, and samples were centrifuged at 2500 rpm for 10 minutes and the serum separated. For the APST, citrated blood was centrifuged at room temperature to separate the plasma. Afterwards, patients received intradermal injections of 50 µL of fresh undiluted autologous serum and 50 µL of autologous plasma on the 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/APST was considered when the diameter of serum-induced wheal was >1.5 mm compared to the saline-induced response at 30 minutes.

**Statistical analysis**

Descriptive statistics were performed for each variable, using median, range and percentiles 25th (P25) and 75th (P75) for quantitative variables, and absolute (n) and relative (%) frequencies for categorical variables. Mann-Whitney U test was used to compare the FcεRI receptor expression between patients with CSU, CIndU and HCs. Pearson’s correlation was used to evaluate the association of FcεRI receptor expression with serum IgE levels, blood basophil count, disease duration and scores of disease severity. Paired samples T-test and Wilcoxon signed-rank test were used to evaluate changes in FcεRI levels during treatment with antihistamines and omalizumab, respectively. All analyses were carried out with the SPSS 22.0 statistical package, and a p-value < 0.05 was considered statistically significant.
RESULTS  
Demographics and FcεRI expression in the study population  
During the study period, 287 CU patients were referred to our Clinic and were therefore included in the analysis. Of these, 192 (66.9%) patients suffered from CSU predominantly and the remaining 95 (33.1%) from pure CIndU (54 cold urticaria, 15 symptomatic dermographism, 10 solar urticaria, 7 cholinergic urticaria, 7 delayed pressure urticaria, 1 contact urticaria and 1 aquagenic urticaria). In addition, 46 HCs were included to obtain reference data. Clinical and demographics features of the study population are summarized in Table I.  
Regarding basophil FcεRI levels, the median (P25-P75) FcεRI expression was found significantly higher in CU patients compared to HCs (9033 [5864- 13630] of MFI vs. 4743 [2771- 7580] of MFI, respectively; p< 0.0001; Figure 1). However, among CU patients, no significant differences regarding the FcεRI expression were found in patients with CSU compared to those with pure CIndU (9234 [5934- 13534] of MFI vs. 8932 [5566- 13919] of MFI, respectively; p= 0.826; Figure 1). It should be also mentioned that no significant differences were observed regarding total serum IgE levels and basophil count between these two groups of patients (CSU vs. pure CIndU; Table I). However, CU subjects showed significantly lower blood basophil numbers than HCs (p= 0.005).  
FceRI expression and clinical and laboratory variables  
Some clinical and laboratory variables had significant association with basophil FcεRI expression in our cohort of CU patients. Thus, subjects who presented with concomitant atopic features showed significantly higher FcεRI levels than those without personal history of atopic dermatitis, allergic rhinitis and/or allergic asthma (median
FcεRI expression: 11534 [6561-15649] of MFI vs. 8583 [5438- 13109] of MFI, respectively; \( p = 0.003 \). Likewise, higher basophil FcεRI levels were detected in CSU patients with negative ASST compared to those with positive ASST (median [P25-P75] FcεRI expression: 10684 [7352-16150] of MFI vs. 8061 [1301- 12726] of MFI, respectively; \( p = 0.002; \) Figure 2). A similar trend was found regarding the APST result (median [P25-P75] FcεRI expression: 10403 [6992-15515] of MFI in APST negative patients vs. 7903 [1243- 13601] of MFI in APST positive patients; \( p = 0.009; \) Figure 2).

FcεRI expression also differed among CU patients depending on the levels of circulating ATAs, with lower FcεRI levels in patients with elevated ATA levels (i.e. >35 UI/mL of anti-TPO and/or >40 UI/mL of anti-Tg; median [P25-P75] FcεRI expression: 6442 [1621-11141] of MFI vs. 9396 [6261-13793] of MFI, \( p = 0.010; \) Figure 2).

We also investigated whether the basophil FcεRI expression in CU patients could be associated with total serum IgE levels and/or blood basophil count. A positive correlation was found between IgE levels and the FcεRI expression (\( R = 0.422; \) \( p < 0.001; \) Figure 3). Conversely, no association was detected between blood basophil count and FcεRI levels (\( R = 0.095; \) \( p = 0.132 \)).

**FcεRI expression and disease activity and duration**

Disease activity was evaluated by using the UAS7 in CSU patients and appropriate threshold tests in CIndU patients (e.g. the critical temperature threshold [CTT] and the critical stimulation time threshold [CsTT] assessed by the TempTest\textsuperscript{®} 3.0 in patients with cold urticaria).\textsuperscript{15-17} In this case, the basophil FcεRI expression was not found associated with disease activity in CSU patients (\( R = 0.114; \) \( p = 0.156 \)), or with the CTT (\( R = 0.062; \) \( p = 0.708 \)) and the CsTT (\( R = 0.010; \) \( p = 0.953 \)) in patients with cold urticaria.
urticaria. Regarding CU prognosis, disease duration, defined as the time from symptoms onset to the initial evaluation, was also not found associated with the basophil FceRI expression in CU patients (R= 0.031; p= 0.613).

FceRI expression and therapeutic response

FceRI levels were evaluated in 60 subjects (47 CSU and 13 CIndU) during antihistamine therapy (median [range] number of months on therapy before follow-up measurement: 3 [1-21] months). In this group of patients, FceRI expression was not significantly modified during treatment (p= 0.118; Figure 4a). Furthermore, no significant differences were observed regarding the baseline FceRI expression in responders and non-responders to antihistamines (p= 0.787). On the other hand, in the 14 patients diagnosed with pure CIndU who received treatment with omalizumab (9 cold urticaria, 3 solar urticaria and 2 symptomatic dermatographism), a significant drop in the basophil FceRI expression was observed after the first injection (median [P25–P75] reduction from baseline at 4 weeks: 86.4% [83.7–93.6]; p= 0.003; Figure 4b), and such reduction was maintained throughout the whole treatment (median reduction from baseline at weeks 8 and 20: 90.3% and 88.0% respectively). At 6 months of anti-IgE therapy, 11 (78.6%) patients achieved significant clinical improvement (UCT ≥12), while 3 (21.4%) subjects were considered to have poorly controlled disease (UCT <12). Interestingly, these omalizumab “non-responders” showed very low baseline FceRI levels (median [range] FceRI expression: 2547 [1172-3778] of MFI in “non-responders” vs. 13591 [7982-18512] of MFI in “responders”).

DISCUSSION
FcεRI is a molecular complex expressed on the surface of mast cells, basophils, antigen-presenting cells and eosinophils, and its activation appears to be critical for the immediate hypersensitivity response that is characteristic of allergic diseases.\textsuperscript{18} Thereby, FcεRI expression has been found significantly upregulated in subjects with atopic dermatitis, allergic asthma and allergic rhinitis compared to healthy non-atopic individuals.\textsuperscript{19,20} Likewise, the results obtained from the present large cohort study demonstrate that FcεRI expression on circulating basophils is also substantially increased in patients with active CSU and CIndU. Although the exact functional significance of elevated FcεRI expression on effector cells in allergic conditions is not completely understood, accumulated evidence suggests that these receptors could enhance their roles as effector cells in allergic inflammation.\textsuperscript{19} It has been also postulated that elevated FcεRI expression might profoundly alter the spectrum of allergen-presenting cells available to present allergens to T cells,\textsuperscript{20} and that FcεRI down-regulation may be followed by an increase in the threshold above which degranulation of effector cells is triggered.\textsuperscript{13} Taken together, our observations support the involvement of FcεRI on the complex inflammatory response that occurs in patients with CSU and CIndU, and also support the assumption that circulating basophils play an important role in the pathophysiology of CU.

Several lines of evidence also support a regulatory role for serum IgE in the expression of its high-affinity receptor on human mast cells and basophils.\textsuperscript{21} Thereby, a very strong association (correlation coefficient close to 1) has been found between serum IgE levels and FcεRI expression on effector cells in a great variety of disease states, particularly in atopic individuals, but also in other IgE-driven conditions like hyper-IgE syndrome or helminth infestation.\textsuperscript{19,22} Although the basis for this correlation has not been elucidated in detail, it has been suggested either that there are similar
regulatory mechanisms to both IgE levels and IgE receptor or that IgE itself upregulates or stabilizes surface expression of the receptor leading to elevated expression in allergic diseases. However, such association has not been previously evaluated in patients with CSU and CIndU. According to our results, in these conditions, which are not considered classic allergen-driven diseases, this potential association IgE- FcεRI expression seems to be weaker (R=0.422), suggesting that there must be other regulatory mechanisms with a significant influence on the FcεRI levels in CU patients. In addition, it does not appear that certain CU features, such as the activity/severity or the duration of the disease, may play an important role in the regulation of FcεRI expression on effector cells.

Previous studies have indicated that approximately 30-60% of CU patients may have an autoimmune etiology on the basis of various laboratory and clinical evidence. Such autoimmune background is supported by the identification of circulating autoantibodies against FcεRI or (less commonly) IgE that may induce activation of basophils and mast cells, secretion of histamine and recruitment of inflammatory cells. The detection of such autoantibodies may have a complex methodology with variable sensitivity and specificity and, as of yet, is not fully implemented in routine clinical practice. Accordingly, several tests have been proposed to evaluate such autoimmune mechanism in CU patients. Among them, two of the most accessible and used in daily practice are the ASST/APST and the detection of circulating ATAs, since accumulated evidence demonstrates that autoantibodies to FcεRI are more frequently identified in patients with positive ASST and/or elevated ATA levels, which may be in line with our findings. Thus, according to our results, patients with negative ASST/APST or undetected ATAs showed significantly higher basophil FcεRI levels, suggesting that these autoantibodies against FcεRI (or other...
functional autoantibodies that bind to the FcεRI receptor) that are presumably present in patients with autoimmune CU could interfere in the measurement of the basophil FcεRI expression, reducing their levels detected by flow cytometry. Thereby, although there was an overlap of values between both group of patients, it could be said that the assessment of the basophil FcεRI expression may help distinguishing CU individuals according to the potential pathogenic mechanism of their disease. These observations could have important implications for clinicians, since CU patients with an autoimmune etiology may present distinctive clinical features and patterns of therapeutic response. Further research is needed to see whether the FcεRI measurement can distinguish autoimmune patients without this overlap.

Given the emergence of new therapies in CU, there is a growing interest to look for objective biomarkers that reliably predict the effectiveness of a specific therapeutic intervention. Thus, many clinical and laboratory parameters have been claimed to correlate with the response to therapy in CU during the last years. However there are no validated biomarkers in clinical practice for this disease to date. In the particular case of omalizumab, previous data from our group and Metz et al demonstrated that the fast clinical improvement achieved during omalizumab therapy in CSU patients is associated with a rapid and sharp reduction in the FcεRI expression on effector cells that is maintained throughout the whole treatment. Furthermore, recent investigations have shown that CSU patients who do not respond to omalizumab therapy have lower baseline FcεRI levels on basophils compared to responder patients, suggesting that this biomarker may represent a potential predictor of omalizumab response in CSU. Similarly, in the present study, we have observed that this trend can also be applied in CIndU patients, since non-responders to omalizumab showed almost undetectable baseline FcεRI levels. The reason why these patients show very low basophil FcεRI
expression is not yet fully understood. Genetic variability or some masquerading factors could be plausible explanations, since previous studies have shown that experimental molecular changes in the subunits of this receptor can affect its expression on the cell surface.\textsuperscript{33} Although further multicentre prospective studies are needed to confirm such observations, these findings might have an important impact in daily practice, allowing physicians to early identify patients who will not benefit from omalizumab therapy.

Regarding antihistamines, previous data have also suggested the existence of a possible link between certain aspects related to the IgE receptor and the response to this treatment in CU patients. Thus, for example, Guo et al identified a single nucleotide polymorphism in the FCER1A gene (a gene that encodes the \( \alpha \)-chain of the FceRI) that might be associated with the therapeutic efficacy of non-sedating antihistamines in Chinese patients with CSU.\textsuperscript{34} However, to our knowledge, the effect of antihistamine therapy on the FceRI expression has not been previously evaluated. In this sense, we could not find an association between FceRI levels and response to antihistamine therapy in our cohort of CU patients, since basophil FceRI expression was not significantly modified during treatment and no differences regarding this parameter were observed between responder and non-responder patients.

Some limitations of the present study should be pointed out. The analysis was done based in data from real clinical practice and therefore, some comparisons may not have reached statistical significance probably due to a small sample size and insignificant power to show statistical differences (a prior sample calculation was not addressed). A matching procedure for some variables (e.g. age and sex) was not used for the selection of the control group. The overlap of 25-75 ranges between ASST/APST positive (autoimmune) and negative patients, even though the median values were significantly different, may limit the blood basophil FceRI expression
clinical relevance. Further studies in larger patient populations are needed to see how
distinct the two populations are, to determine the amount of overlap and to establish a
cut-off value for FcεRI expression that might provide an optimal sensitivity and
specificity. Although the evolution of the basophil FcεRI expression appears to be
similar as in CSU patients, the limited number of patients with CIndU treated with
omalizumab in the present study does not allow drawing firm conclusions about the
baseline FcεRI expression as a biomarker of omalizumab response.

In summary, FcεRI expression on blood basophils is significantly upregulated in
subjects with CSU and CIndU compared to healthy non-atopic individuals. The
association between serum total IgE levels- basophil FcεRI expression in CU is weaker
than in other classic allergic diseases, suggesting that there must be other regulatory
mechanisms with an important influence on the FcεRI levels in this disease.
Furthermore, significantly higher FcεRI levels might be detected in CU patients who
present with concomitant atopic features, negative ASST/APST or undetected levels of
ATAs. Although FcεRI expression would not provide information regarding CU
activity and prognosis, its assessment might be relevant in daily clinical practice,
helping physicians to identify those patients with the suggested autoimmune
pathogenesis (driven by IgE or IgG autoantibodies against FcεRI). More extensive
studies would be useful to confirm this observation and to define the clinical value of
FcεRI expression in blood basophils as a potential biomarker of omalizumab response.
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REFERENCES


**FIGURE LEGENDS**

**Figure 1:** Box-whiskers plots presenting median, interquartile range and maximum and minimum of basophil FceRI levels in all CU patients (n=287), patients with CSU (n=192), patients with CIndU (n=95) and healthy controls (n=46). ***p < 0.0001

**Figure 2:** Box-whiskers plots presenting median, interquartile range and maximum and minimum of basophil FceRI levels in CU patients with positive and negative results of the autologous serum skin test (ASST) and the autologous plasma skin test (APST) and elevated and non-elevated levels of anti-thyroid antibodies (ATA).

**Figure 3:** Correlation between basophil FceRI expression and total serum IgE levels [represented in logarithmic scale] in CU patients.

**Figure 4:** Evolution of the basophil FceRI expression (median, interquartile range) during treatment with (A) antihistamines and (B) omalizumab in CU patients.
Supplementary Figure 1: Comparison of the basophil gating strategy. (A) Anti-CD123-PE and (C) anti-CD193-APC allows the identification of basophils among the cells contained in the respective gate. (B & D) High-affinity IgE receptor expression on basophils gated in A and C, respectively.
Table I. Clinical and demographic features of the study population.

<table>
<thead>
<tr>
<th>Clinical variables</th>
<th>CU patients (n= 287)</th>
<th>CSU patients (n= 192)</th>
<th>CIndU patients (n= 95)</th>
<th>Healthy controls (n= 46)</th>
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<td>Female sex, n (%)</td>
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<td>46 (24.0)</td>
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<td>Thyroid impairment, n (%)</td>
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<td>28 (14.6)</td>
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<td>Elevated levels of ATAs, n (%)</td>
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<td>27 (14.1)</td>
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<td>Disease duration, months (range)</td>
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<td>Median value of CTT, °C (range) ††</td>
<td>NA</td>
<td>NA</td>
<td>14 (4-26)</td>
<td>-</td>
</tr>
<tr>
<td>Concomitant CIndU, n (%)</td>
<td>NA</td>
<td>57 (29.7)</td>
<td>NA</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Positive ASST, n (%) **</td>
<td>NA</td>
<td>78 (62.9)</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>Positive APST, n (%) **</td>
<td>NA</td>
<td>56 (45.2)</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>Total serum IgE levels, kU/L (range)</td>
<td>100.0 (1.0-4700.0)</td>
<td>103.0 (1.0-1855.0)</td>
<td>78.9 (2.6-4700.0)</td>
<td>-</td>
</tr>
<tr>
<td>Blood basophil count, 10³/μl (range)</td>
<td>0.03 (0.01-0.14)</td>
<td>0.03 (0.01-0.14)</td>
<td>0.04 (0.01-0.10)</td>
<td>0.05 (0.02-0.09)</td>
</tr>
</tbody>
</table>

Abbreviations: ASST, autologous serum skin test; APST, autologous plasma skin test; ATAs, anti-thyroid antibodies; CIndU, chronic inducible urticaria; CsTT, critical stimulation time threshold; CSU, chronic spontaneous urticaria; CTT, critical temperature threshold; CU, chronic urticaria; IgE, immunoglobulin E; NA, not applicable; UAS7, 7-day urticaria activity score.
† Defined as the alteration of the thyroid-stimulating hormone serum levels
‡‡ With respect to the 54 patients with cold urticaria
** For clinical reasons, ASST and APST were only assessed in 124 CSU patients
Figure 3

Levels of basophil FcεRI expression (MFI) vs. Total serum IgE (kU/L) [log scale].

$r = 0.422$

$p < 0.001$
Figure 4

A. Levels of basophil FcεRI expression (MFI) at baseline evaluation and during antihistamine therapy for responders and non-responders.

B. Levels of basophil FcεRI expression (MFI) over time after the first dose of omalizumab for responders and non-responders.