

Histamine down-regulates the FCER1 α -chain expression in human IL-4-activated M2 macrophages

To the Editor,

The high-affinity Fc receptor for IgE (FCER1) is best known for its crucial role in allergic mediated reactions. FCER1 is highly expressed on mast cells and basophils and captures IgE in its monomeric form.

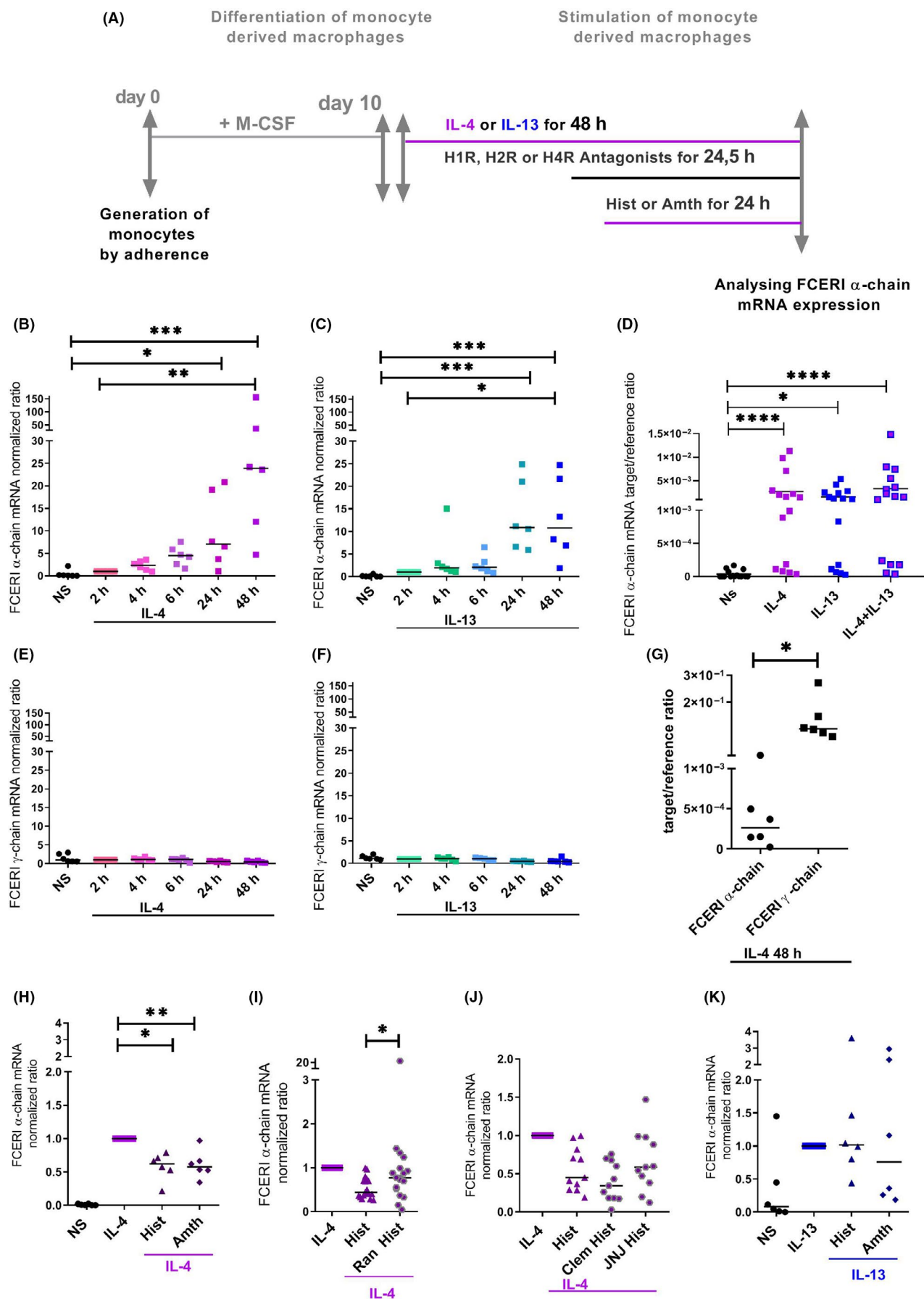
In contrast to mice in humans, the expression of the FCER1 is not restricted only to mast cells and basophils. The trimeric form of the FCER1 on monocytes, DCs and myeloid cells is comprised of one α -subunit and the two γ -subunits. Interestingly, the FCER1 was first found in human monocytes derived from patients with allergic diseases.¹ Later, it was shown that the receptor is also expressed on monocytes from healthy donors and prevents apoptosis in these cells.²

Alternative activation of macrophages is mediated via IL-4 and IL-13 acting through their common receptor chain IL-4R α . These so-called M2a macrophages perform important active and regulatory functions in innate as well as in adaptive immunity.³ The regulation of the FCER1 expression on human M2 macrophages has not been investigated in detail, in particular under allergic conditions, until yet. It was shown that crosslinking of the FCER1 on monocytes leads to H₁R-expressing CD68-positive macrophages which accumulate in the dermal compartment of the skin of atopic dermatitis⁴ where

histamine is present in high concentrations. Monocyte-derived M2 macrophages differentiated in the presence of M-CSF for 10 days showed an up-regulation of the differentiation marker CD68 and expression of H₁R, H₂R, and H₄R (but not of H₃R).⁵

In this study, we investigated the expression of the FCER1 α -chain on human M2 macrophages in response to IL-4 or IL-13 and histamine. We differentiated M2 macrophages for 10 days showing expression and up-regulation of the co-stimulatory molecule CD86 by IL-4 and IL-13. CD80 was not expressed. The M2 marker CD206 was expressed on M2 macrophages and was up-regulated by IL-4 or IL-13, whereas the M2 marker CD163 which was also expressed on most M2 macrophages (up to 87%) was slightly down-regulated by Th2 cytokines (Figure S1). Similar phenotypes for M2 macrophages were described by Pellizzari et al.⁶ After stimulation with IL-4 (20 ng/mL) or IL-13 (15 ng/mL) for different time periods as indicated in Figure 1B,C and Figure 1E,F, FCER1 α -chain and FCER1 γ -chain mRNA expressions were analyzed by RT-PCR. We observed a step-wise up-regulation of FCER1 α -chain mRNA expression by time in response to IL-4 or IL-13 which was most pronounced after 48 hours (Figure 1B,C). Simultaneous stimulation with both cytokines for 48 hours showed a moderate additive effect when compared to the

FIGURE 1 IL-4 or IL-13 up-regulates FCER1 α -chain mRNA expression in human M2 macrophages. The IL-4-induced FCER1 α -chain mRNA expression is down-regulated by histamine. A, Primary human monocytes obtained from buffy coats were differentiated into M2 macrophages (M-CSF, 10 ng/mL) for 10 d. After differentiation, M2 macrophages were stimulated with or without IL-4 (20 ng/mL) or IL-13 (15 ng/mL). After the incubation time of 24 h, the IL-4- or IL-13-stimulated cells were stimulated with or without histamine or amthamine. Antagonists of the different histamine receptors were added ½ h before the addition of histamine to the cells. Twenty-four h later, all samples were lysed and RNA was collected. According to this protocol, the cells were cultured in the presence of IL-4/IL-13 for 48 h and in the presence of histamine and IL-4/IL-13 with or without antagonists for 24 ½ or 24 h, respectively. B, FCER1 α -chain mRNA expression in IL-4-stimulated M2 macrophages. C, FCER1 α -chain mRNA expression in IL-13-stimulated M2 macrophages. D, mRNA target/reference ratios of FCER1 α -chain mRNA expression in IL-4 or IL-13 or in IL-4- and IL-13-stimulated M2 macrophages. E, FCER1 γ -chain mRNA expression in IL-4-stimulated M2 macrophages. F, FCER1 γ -chain mRNA expression in IL-13-stimulated M2 macrophages. G, mRNA target/reference ratios of FCER1 α -chain and FCER1 γ -chain mRNA expression in M2 macrophages activated with IL-4 for 48 h. FCER1 α -chain mRNA expression in IL-4-activated M2 macrophages stimulated H, with histamine or amthamine (H2R agonist) I, with ranitidine (H2R antagonist) + histamine J, with clemastine (H1R antagonist) + histamine or JNJ7777120 (H4R antagonist) + histamine (10 μ mol/L each ligand). Blockade of histamine receptors is shown in experiments with a down-regulation of the FCER1 α -chain mRNA expression by histamine. K, FCER1 α -chain mRNA expression in IL-13-activated M2 macrophages stimulated with histamine or amthamine (H2R agonist). FCER1 α -chain and FCER1 γ -chain mRNA expressions were detected by quantitative PCR and calculated by the $[\Delta\Delta Ct]$ method. In B, C, E, and F, the expression of FCER1 α -chain or FCER1 γ -chain mRNA was normalized to the samples which were stimulated for 2 h with IL-4 (some nonstimulated samples did not express FCER1 α -chain mRNA). In D and G, target/reference ratio is depicted, and in H-K, the expression of FCER1 α -chain mRNA was normalized to the IL-4- or IL-13-stimulated samples. Significant differences, as determined by Friedman Dunn's multiple comparison test in B, C, and D, by Friedman Dunn's multiple comparison test selected pairs in H, and by the Wilcoxon matched-pairs signed-rank test in G and I (Hist vs Ran + Hist), are indicated as follows: * $P < .05$; ** $P < .01$; *** $P < .001$; and **** $P < .0001$; medians are shown in the graphs. B, C, E, F, G, H, and K ($n = 6$ independent donors and experiments), D ($n = 15$ independent donors and experiments), I ($n = 18$ independent donors and experiments), and J ($n = 11$ independent donors and experiments). NS, nonstimulated; Hist, histamine; amthamine, Amth; Ran, ranitidine; Clem, clemastine; JNJ = JNJ7777120



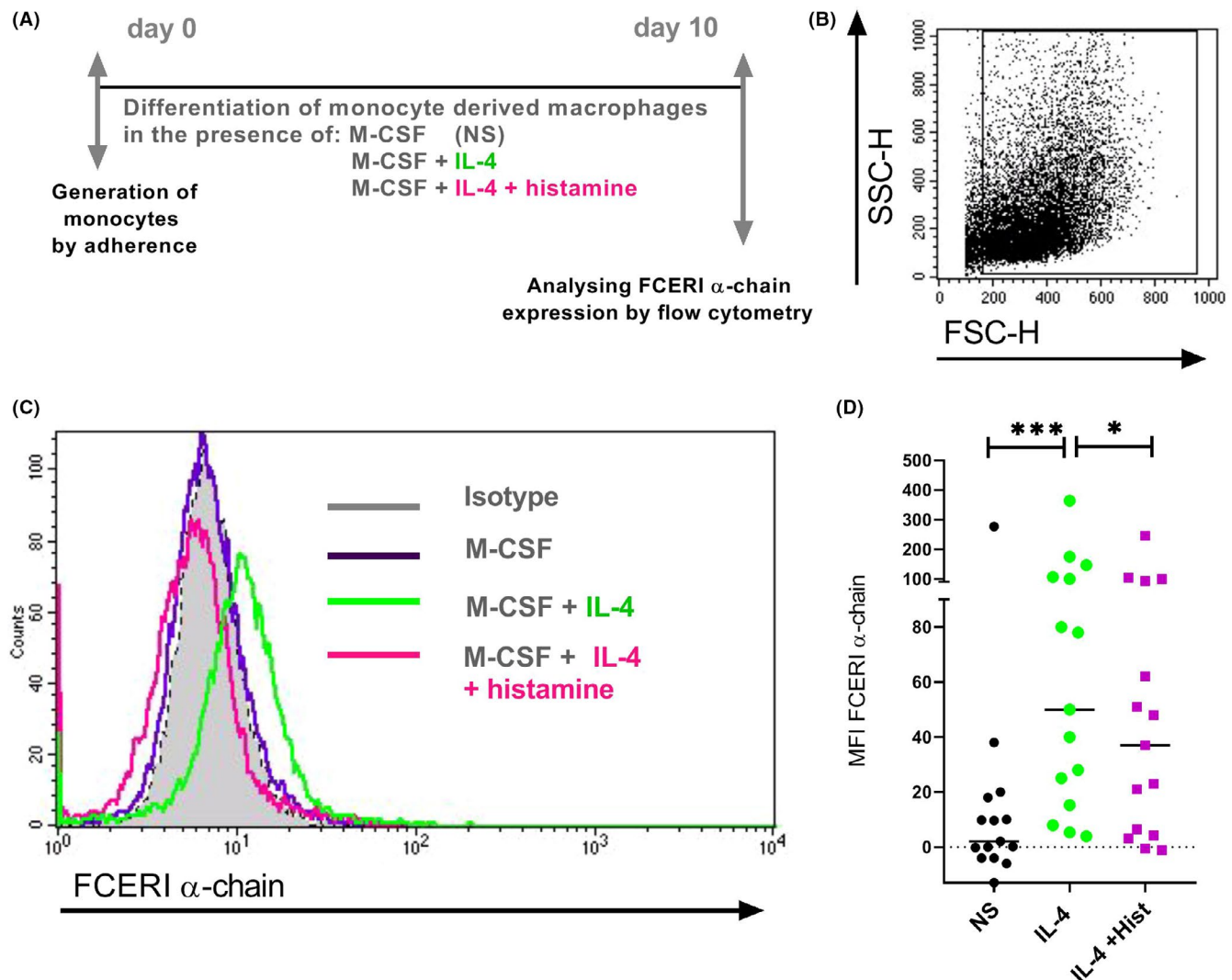


FIGURE 2 Histamine down-regulates the IL-4-induced intracellular expression of the FCER1- α in M2 macrophages. A, M2 macrophages which were differentiated: (a) in the presence of M-CSF only, (b) in the presence of M-CSF + IL-4, and (c) in the presence of M-CSF + IL-4 + histamine for 10 days. After 10 days, Fixable Viability Dye staining was performed before cell fixation/permeabilization and specific staining. The gate was placed on the living cells during flow cytometry analysis, and this population was analyzed in specific stains. The cells of each group were then stained intracellularly with a mouse anti-human FCER1 α -chain IgG2b antibody and the matched isotype control. The expression of the FCER1 α -chain was investigated by flow cytometry. The histamine effect is shown in experiments with an up-regulation of the FCER1 α -chain expression by IL-4. B, Macrophages were gated by forward and sideward scatter. C, One representative histogram out of the 15 experiments which are shown in D. Significant differences, as determined by Friedman Dunn's multiple comparison test selected pairs in D, are indicated as follows: * $P < .05$; *** $P < .001$; medians are shown in the graphs. D, (n = 15) independent donors and experiments). NS, nonstimulated, Hist, histamine

IL-4- or IL-13-stimulated cells (Figure 1D). The FCER1 γ -chain mRNA was higher expressed when compared to the target/reference ratios of the FCER1 α -chain mRNA expression (Figure 1G). However, neither IL-4 nor IL-13 influenced the mRNA expression of the FCER1 γ -chain (Figure 1E,F).

Pellizzari et al described highest cell surface expression of the FCER1 on M2 macrophages compared to monocytes or to M1 macrophages. However, the regulation of FCER1 α -chain expression was not directly related to IL-4 stimulation in their study.⁶

Thus, we show here for the first time that IL-4 and also IL-13 up-regulate FCER1 α -chain mRNA expression in human M2 macrophages.

M2 macrophages first activated by IL-4 or IL-13 for 24 hours were subsequently stimulated with histamine, or with the H2R agonist amthamine or pre-incubated with antagonists for H1R (clemastine), H2R (ranitidine), or H4R (JNJ7777120) (10 μ mol/L each ligand) for further 24 hours as indicated in Figure 1H-J. Remarkably, in contrast to IL-13 (Figure 1K) the IL-4-induced up-regulation of FCER1 α -chain mRNA expression was significantly down-regulated by histamine and amthamine (Figure 1H). Pre-incubation of the cells with the H2R antagonist ranitidine reversed the histamine-mediated down-regulation of FCER1 α -chain mRNA expression (Figure 1I), whereas H1R and H4R (Figure 1J) antagonists showed no effect.

In contrast to the effects of histamine or the H2R agonist showing a consistent down-regulation of FCER1 α -chain mRNA, 2-pyr-dylethylamine, (H1R agonist), 4MH (4MH) (H2R/H4R agonist), and ST-1006 (H4R agonist) showed a down-regulation of the FCER1 α -chain mRNA expression only in some donors but these effects did not reach statistical significance (Figure S2B-D).

M2 macrophages were differentiated: (a) in the presence of M-CSF only, (b) in the presence of M-CSF + IL-4, and (c) in the presence of M-CSF + IL-4 + histamine during the whole differentiation process of 10 days (Figure 2A). After 10 days, M2 macrophages of each group were stained intracellularly with an anti-human FCER1 α -chain IgG2b antibody and the matched isotype control. We observed that the constitutive intracellularly localized FCER1 α -chain was very low or not expressed. IL-4 induced an up-regulation of the FCER1 α -chain expression intracellularly which was down-regulated by histamine (Figure 2C,D).

The up-regulation of intracellular expression of the FCER1 α -chain by IL-4 in human M2 macrophages in our study confirms observations made in dendritic cells (DCs) where IL-4 up-regulated the intracellular endosomal expression of the FCER1 α -chain but the stimulation with IL-4 was not sufficient to induce its expression at the cell surface of DCs.⁷

The down-regulation of the FCER1 α -chain mRNA and its intracellular expression mainly via H2R supports the observation that the H2R promotes anti-inflammatory immune responses as previously shown by its capacity to inhibit TLR-induced up-regulation of cytokines of the IL-12 family in monocytes or dendritic cells.⁸

Our novel findings provide further hints for a role of histamine to prevent excessive IgE dependent mechanisms not only by limiting mediator release from basophils as shown in a previous publication⁹ but also by down-regulating the IL-4-induced FCER1 α -chain expression levels in human M2 macrophages. The histamine-mediated down-regulation of the FCER1 α -chain expression in IL-4-activated M2 macrophages may reduce IgE/FCER1 antigen sensing and signal transduction thereby balancing the inflammatory conditions in allergic reactions. These observations may have an impact on the treatment of allergic diseases.

KEYWORDS

histamine, high-affinity Fc receptor for IgE, inflammation, macrophages

CONFLICT OF INTEREST

Susanne Mommert has nothing to disclose. Katrin Schaper-Gerhardt reports grants from Deutsche Forschungsgemeinschaft, during the conduct of the study. Judith Tabea Schaper has nothing to disclose. Ralf Gutzmer reports grants from Deutsche Forschungsgemeinschaft, during the conduct of the study; personal fees and nonfinancial support from Bristol Myers Squibb, personal fees and nonfinancial support from Roche Pharma, personal fees and nonfinancial support from Merck Serono, grants, personal fees and nonfinancial support from Amgen, personal fees and nonfinancial support from Pierre Fabre, personal fees and

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ETHICAL APPROVAL

The investigation of the role of the histamine receptors in inflammatory diseases was approved by the local ethics committee of the Hannover Medical School (Vote 4253) and was conducted according to the Declaration of Helsinki Principles. All participants in the study including healthy control persons gave their written informed consent. The informed consent for human participants under the age of 18 years was obtained from a parent and/or legal guardian. The study was approved by the local ethics committee and was in accordance with the protocols of the Declaration of Helsinki.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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A very low number of circulating basophils is predictive of a poor response to omalizumab in chronic spontaneous urticaria

To the Editor,

Chronic spontaneous urticaria (CSU) is characterized by the spontaneous appearance of wheals, angioedema or both for more than 6 weeks.¹ CSU signs and symptoms, albeit not life-threatening, markedly affect patients' quality of life.¹ Mast cell activation and degranulation with subsequent release of inflammatory mediators play a key role in CSU symptom development; however, several points of evidence suggest that basophils also contribute to the disease.^{1,2} Omalizumab, a recombinant humanized monoclonal anti-IgE antibody, represents an effective treatment of CSU and is often used in patients unresponsive to high doses of H1 antihistamines.¹ However, the response to treatment is variable, as some CSU patients respond to treatment within days of their first omalizumab injection, others respond within weeks, and a subgroup of patients do not respond to omalizumab at all.^{1,2} The aim of this study was to evaluate the predictive value of basophil-associated markers, measured before starting omalizumab treatment, for the response to omalizumab in patients with CSU.

In this prospective study, we enrolled 43 patients with active CSU referred to the University Clinic of Respiratory and Allergic Diseases Golnik (Slovenia), whose symptoms were not controlled with high doses of H1 antihistamines and were consequently treated with omalizumab. Their median age was 48 (range, 22-81) years, 70% were women, and the median urticaria activity score 7 (UAS7) before the start of omalizumab treatment was 28 (range, 12-42). The demographic, clinical and laboratory features of CSU patients are

summarized in Table 1. The study was approved by the Slovenian National Medical Ethics Committee (KME78/09/14), and all patients gave their informed consent. With the exception of two patients who received 150 mg, all patients received 3 subcutaneous doses of 300 mg omalizumab at 4-week intervals. Response to treatment was defined as a sustained improvement of CSU symptoms (UAS7 ≤6) within 2 weeks (early complete responders) or 12 weeks (late complete responders), while no response (NR) was defined as no improvement or only minor improvement of CSU symptoms within 12 weeks. Before the start of omalizumab treatment, total serum IgE; absolute cell count of basophils, lymphocytes, monocytes and granulocytes; CD63 basophil activation and CD63 activation of donor basophils after stimulation with sera of CSU patients; FcεRI and IgE quantification on the basophil surface; and whole-blood gene expression of basophil-related markers (*FCER1A*, *CPA3* and *HDC*) were determined as previously described.^{3,4} The basophil gene mean was calculated as the mean of standardized gene expression variables of *FCER1A*, *CPA3* and *HDC*. For detailed overview of study methodology, refer to Appendix S1.

The rate of clinical response to omalizumab treatment was 84% (36/43). Among CSU responders (CSU-R), 23 (53%) patients were classified as early complete responders and 13 (30%) as late complete responders. Seven (16%) patients were nonresponders (CSU-NR) (Figure S1). No significant differences in age, gender distribution, body mass index (BMI), disease duration or baseline UAS7 were found between CSU-R and CSU-NR (Table 1).