

SHORT REPORT

Omalizumab may not inhibit mast cell and basophil activation *in vitro*

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Abstract

Background In March 2014, omalizumab, a monoclonal anti-IgE antibody, was approved for the treatment of chronic spontaneous urticaria (CSU). The primary mode of action of omalizumab is considered to be the reduction in free IgE serum levels and the subsequent down-regulation of FcεRI, the high affinity receptor for IgE, on mast cells and basophils. Recently, it has been suggested that most CSU patients have an autoimmune aetiology which may lead to chronic activation of mast cells and basophils.

Objective To understand more of the mechanisms by which omalizumab may exert its effects in CSU, its efficacy was tested on human mast cells and basophils.

Methods Omalizumab, which was or was not preincubated with serum from healthy donors or CSU patients, was coin-cubated with isolated healthy donor skin mast cells or peripheral blood-derived monocytes containing 1–2% basophils. Degranulation was induced using anti-human IgE, C5a, or substance P and histamine release determined.

Results Anti-human IgE-induced histamine release from mast cells or basophils was not altered in the presence or absence of omalizumab. In contrast, preincubation of mast cells with DARPin Fc fusion protein, a positive control for negative signalling via FcεRI-FcγRIIb cross activation, significantly diminished histamine release. Moreover, omalizumab, that was preincubated with healthy donor serum, CSU patient serum or auto-reactive CSU serum to allow for the formation of potential immune complexes, did not alter induced histamine release in a coin-cubation setup with mast cells or basophils as compared to the absence of omalizumab. *In vivo*, blood basophil numbers and basophil histamine content increase under omalizumab therapy.

Conclusion Our results suggest that the rapid response to omalizumab therapy is more likely to result from the elimination of an activating signal rather than the generation of a negative, inhibitory signal.

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Conflicts of interest

The authors declare no conflict of interest.

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Introduction

In March 2014, omalizumab, a monoclonal anti-IgE antibody, was approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of chronic spontaneous (idiopathic) urticaria (CSU) following many successful clinical trials and retrospective clinical analyses.^{1,2} Omalizumab was originally introduced for the treatment of severe allergic asthma, but has also been reported to be effective in the treatment of other mast cell-mediated diseases, such as atopic dermatitis and mastocytosis.^{3–5} The primary mechanism of action of omalizumab in these conditions is considered to be the reduction in free IgE serum levels and the subsequent

down-regulation of FcεRI, the high affinity receptor for IgE, on mast cells and basophils. Recently, it has been suggested that most CSU patients have an autoimmune aetiology with some patients producing IgE autoantibodies against autoantigens, such as thyroperoxidase (TPO), whereas other patients make IgG autoantibodies against the high affinity IgE receptor (FcεRI) and/or IgE, which may chronically activate mast cells and basophils.⁶ Therefore, to understand more of the mechanisms by which omalizumab may exert its effects in CSU, we examined its efficacy on isolated human mast cells and basophils.

In our initial experiments (Fig. 1a), mast cells were freshly isolated from the skin of healthy donors as previously

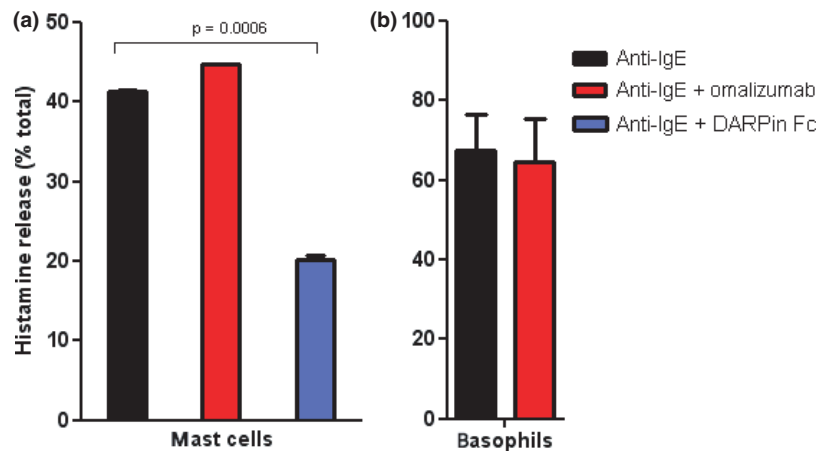


Figure 1 Omalizumab has no inhibitory effect on anti-IgE-induced degranulation. (a) Freshly isolated human skin mast cells were coincubated with or without omalizumab (10 $\mu\text{g/mL}$) for 24 h or with DARPin Fc (100 pmol/mL) for 1 h as positive control for $\text{Fc}\epsilon\text{RI-Fc}\gamma\text{RIIb}$ cross linking. Mast cells were then activated with anti-IgE (1 $\mu\text{g/mL}$) for 1 h and histamine release determined. Results are expressed as mean $\pm$ SEM histamine release (% total) of three independent experiments. Panel (b) shows peripheral blood-derived monocytes from healthy donors that were coincubated with or without omalizumab (10 $\mu\text{g/mL}$) for 1 h and then activated using anti-IgE (5 $\mu\text{g/mL}$) for another hour to induce degranulation. Results are expressed as mean $\pm$ SEM histamine release (% total) of five independent experiments.

described.⁷ Compared with a baseline of $3.0 \pm 0.9\%$ histamine release, mast cells released $41.4 \pm 0.01\%$ of total histamine on stimulation for 1 h with goat anti-human IgE (1 $\mu\text{g/mL}$, KPL, Gaithersburg, USA). Preincubation of mast cells for 1 h with 100 pmol/mL of DARPin Fc fusion protein,⁸ a positive control for negative signalling via $\text{Fc}\epsilon\text{RI-Fc}\gamma\text{RIIb}$ cross activation, significantly ($P = 0.0006$) diminished histamine release. In contrast, preincubation of mast cells for 24 h with 10 $\mu\text{g/mL}$ omalizumab had no effect on anti-IgE-induced mast cell degranulation.

As blood basophils have also been suggested to be involved in the pathogenesis of urticaria,⁹ the effects of omalizumab on these cells were also examined (Fig. 1b). Peripheral blood-derived monocytes containing 1–2% basophils were obtained from healthy donors¹⁰ and activated for 1 h with 5 $\mu\text{g/mL}$ goat anti-human IgE with or without preincubation with omalizumab. In the absence of omalizumab anti-IgE released $67.5 \pm 8.9\%$ of total histamine. Preincubation of peripheral blood-derived monocytes for 1 h with 10 $\mu\text{g/mL}$ omalizumab did not alter anti-IgE-induced histamine release ($64.5 \pm 10.9\%$).

These data indicate that preincubation with omalizumab has no effect on anti-IgE-induced histamine release from primary human mast cells or basophils. Therefore, we next hypothesized that omalizumab may form cell-stabilizing immune complexes after contact with human serum which results in the immediate reduction or even blockade of mast cell and/or basophil activation by cross activation of $\text{Fc}\epsilon\text{RI}$ and the inhibitory $\text{Fc}\gamma\text{RIIb}$.¹¹ To test this hypothesis, we investigated the effects of potential immune complexes derived from coincubation of serum and

omalizumab on the responsiveness of primary skin mast cells from healthy donors.

Skin mast cells were preloaded *ex vivo* with human IgE to mimic the situation present in CSU patients and omalizumab was coincubated with healthy donor serum for 1 h before addition to mast cells for 24 h. However, anti-IgE-induced histamine release did not differ in the absence or presence of omalizumab (Fig. 2a). It is possible that serum of CSU patients contains components which are absent in healthy subjects (e.g. higher levels of IgE) but which might be involved in the formation of immune complexes with omalizumab potentially leading to mast cell stabilization. This prompted us to test the impact of CSU patient serum. However, anti-IgE-induced histamine release following coincubation of mast cells with CSU patient serum (mean total IgE serum level 363.9 ± 374.5 kU/L; range 28.4–892.0 kU/L) for 24 h showed no differences with or without the presence of omalizumab (Fig. 2b). These data indicate that omalizumab does not form mast cell stabilizing immune complexes after contact with healthy or CSU serum. Interestingly, autoantibodies directed against $\text{Fc}\epsilon\text{RI}$, IgE, or TPO are commonly found in at least one-third of CSU patients and are thought to play a role in the pathomechanism of CSU via activation of dermal mast cells.¹² To examine whether the presence of autoantibodies or other autoreactive substances in the serum of CSU patients is required for omalizumab to inhibit mast cell degranulation, we preincubated autoreactive CSU serum (mean total IgE serum level 181.6 ± 138.0 kU/L; range 63.8–367.0 kU/L), which is characterized by inducing $\geq 16.5\%$ histamine release from basophils without further activation¹³, with or without oma-

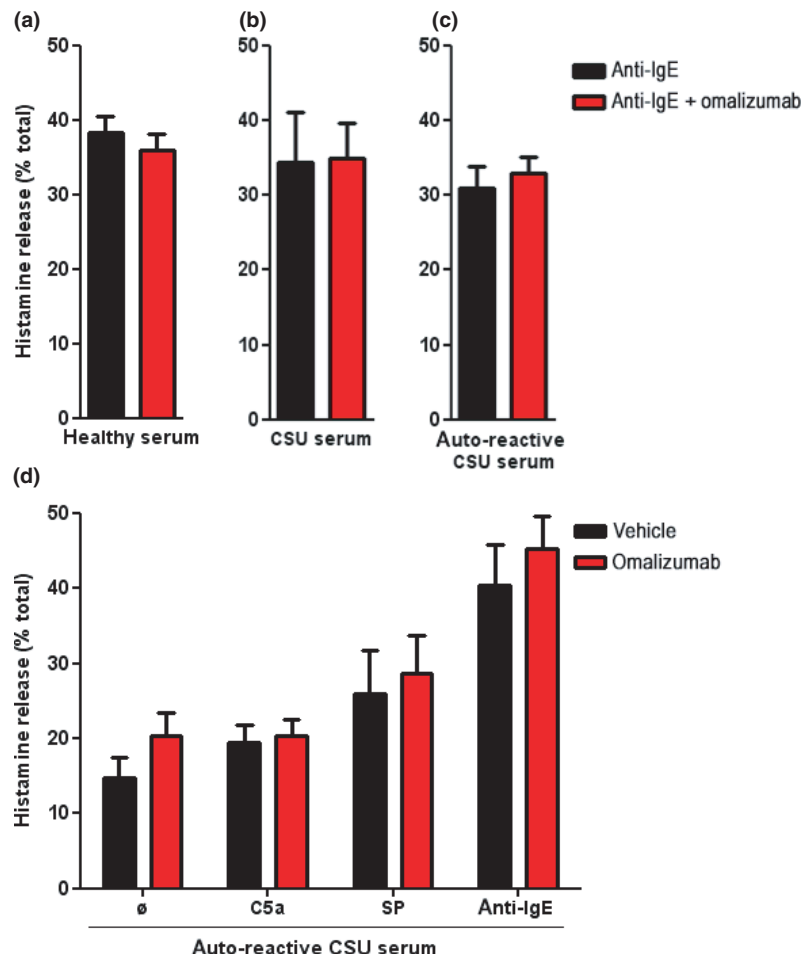


Figure 2 Omalizumab coincubated with human serum does not inhibit anti-IgE-induced degranulation. Aliquots of (a) healthy donor serum, (b) CSU patient serum, and (c) auto-reactive CSU patient serum were preincubated for 1 h with omalizumab (10 μ g/mL) or vehicle (aqua ad iniectionem, B. Braun, Germany) and then coincubated with skin mast cells for 24 h prior to activation with anti-IgE (1 μ g/mL). Histamine release is illustrated as mean $\pm$ SEM (% total) of three independent experiments with $n = 4$ sera per group. Panel (d) shows studies on peripheral blood-derived monocytes containing 1%–2% basophils. Autoreactive CSU serum (characterized to induce $\geq 16.5\%$ histamine release from basophils without further activation) was preincubated for 1 h with omalizumab (10 μ g/mL) or vehicle (aqua ad iniectionem) followed by 1 h coincubation with peripheral blood-derived monocytes. Subsequently, peripheral blood-derived monocytes were not further stimulated ($\emptyset$) or activated with C5a (200 ng/mL), substance P (SP, 10 μ g/mL), or anti-IgE (5 μ g/mL) for 1 h and histamine release was determined as mean $\pm$ SEM (% total). Pooled data of four independent experiments with the same $n = 3$ sera per each group and experiment.

lizumab for 1 h followed by coincubation with human skin mast cells for 24 h. Anti-IgE-mediated activation induced extensive mast cell degranulation, which was not reduced by omalizumab (Fig. 2c). This result indicates that omalizumab does not generate inhibitory signals after getting in contact with auto-reactive CSU serum.

To further test our hypothesis of inhibitory immune complexes potentially formed by omalizumab and CSU serum components with human basophils, we preincubated autoreactive CSU patient serum with omalizumab for 1 h followed

by coincubation with peripheral blood-derived monocytes containing 1–2% basophils for another hour. Autoreactive CSU patient sera were identified beforehand by inducing $\geq 16.5\%$ histamine release from basophils without any further activation. Incubation of peripheral blood-derived monocytes with autoreactive CSU serum alone or further stimulation of these cells using C5a (R&D Systems, Minneapolis, USA), substance P (SIGMA-ALDRICH, Schnelldorf, Germany), or anti-IgE resulted in comparable histamine release regardless of the presence or absence of omalizumab (Fig. 2d). These data

Table 1 Circulating basophil numbers (cells/ μ L) in 14 CSU patients before and after treatment with omalizumab

Number of CSU patients	Baseline before treatment	After 2–4 weeks treatment	Significance
14	10 (10–20)	25 (13–40)	$P = 0.009$

Basophils were counted before treatment (baseline) and after 2–4 weeks treatment. Data are expressed as median (with 25%–75% range) and significance of difference calculated by Student's *t*-test for paired data.

Table 2 *In vitro* basophil histamine release from seven CSU patients (responder) before and after treatment with omalizumab

	Baseline before treatment	After 2–4 weeks treatment	Significance
Total histamine (ng)	41.3 $\pm$ 9.9	72.1 $\pm$ 5.3	$P = 0.031$
Anti-IgE-induced release (%)	30.7 $\pm$ 14.6	47.3 $\pm$ 15.7	$P = 0.356$

Data are expressed as mean $\pm$ SEM and significance of differences calculated using Student's *t*-test for paired data.

indicate that omalizumab exerts no stabilizing effect on human healthy donor basophils after cocubation with healthy or CSU serum.

In our specialist urticaria clinic at the Department of Dermatology and Allergy, Charité – Universitätsmedizin, Berlin, Germany many difficult to treat CSU patients have received therapy with omalizumab during the last years. Clinical progress of these patients was observed during omalizumab treatment, i.e. monitoring disease activity by means of the daily and mean weekly urticaria activity scores (UAS1 and UAS7) to assess patient response to omalizumab. In CSU, “complete response” to omalizumab was defined as a reduction of 90% or more in the UAS7, “significant improvement” as a reduction in the UAS7 of 90%–30% and “no significant improvement” as less than 30% reduction in the UAS7.¹⁴ Numbers of blood basophils were assessed by an automated analyser in the central laboratory of the Charité – Universitätsmedizin Berlin. To assess histamine release from basophils, peripheral blood-derived monocytes containing 1–2% basophils were isolated from fresh heparinized venous blood using the Lymphoprep™ (Axis-Shield PoC AS, Oslo, Norway) density gradient (density 1.077 g/mL, 290 mOsm) containing 9.1% sodium diatrizoate and 5.7% polysaccharide. Cell suspensions were then lysed for total histamine content or were challenged for 30 min at 37°C with anti-IgE (18 ng/mL, KPL) and histamine concentrations were measured. In CSU patients, basopenia is a well-known phenomenon and is suggested to be a result of basophil activation and recruitment into the skin.¹⁵ Interestingly, the number of blood basophils rapidly increased after the first injection of omalizumab therapy (2–4 weeks; Table 1). Moreover, total basophil histamine content significantly ($P = 0.031$) increased 2–4 weeks after injection

of omalizumab as determined for seven CSU patients who had a complete response to omalizumab therapy (Table 2). Although not statistically significant ($P = 0.356$), a tendency of elevated anti-IgE-induced histamine release in these CSU patients (omalizumab responders) was observed (Table 2). Interestingly, basophils from four of these seven CSU patients failed to release histamine before treatment but did so following omalizumab injection. These data indicate that omalizumab treatment does affect patient basophils. However, our *in vitro* studies using freshly isolated human skin mast cells or peripheral blood-derived monocytes in a cocubation setup with serum and omalizumab suggest no involvement of cell stabilizing immune complexes formed by omalizumab and CSU serum components. Indeed, the efficacy of omalizumab seems not to be mediated by direct effects of the drug on mast cells or basophils such as by Fc ϵ RI-Fc γ RIIb cross activation which has been already shown for the anti-IgE DARPin Fc fusion protein.⁸ Recently, the presence of a subset of IgE molecules that can activate mast cells via Fc ϵ RI aggregation in the absence of antigen has been indicated, namely highly cytokinergic IgE.^{16,17} Considering this, it rather appears likely that omalizumab treatment of CSU patients induces a significant drop of free IgE serum levels which in turn may reduce mast cell and basophil activation and degranulation and, finally, CSU symptoms.

In summary, our results suggest that the rapid response to omalizumab therapy is more likely to result from the elimination of an activating signal, such as IgE, rather than the generation of a negative, inhibitory signal. Further *in vivo* and *ex vivo* tests need to be carried out to identify the mechanism of action of omalizumab in CSU.

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