The anti-IgE mAb omalizumab induces adverse reactions by engaging Fcγ receptors

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Omalizumab is an anti-IgE monoclonal antibody (mAb) approved for the treatment of severe asthma and chronic spontaneous urticaria. Use of omalizumab is associated with reported side effects ranging from local skin inflammation at the injection site to systemic anaphylaxis. To date, the mechanisms through which omalizumab induces adverse reactions are still unknown. Here, we demonstrated that immune complexes formed between omalizumab and IgE can induce both skin inflammation and anaphylaxis through engagement of IgG receptors (FcγRs) in FcγR-humanized mice. We further developed an Fc-engineered mutant version of omalizumab, and demonstrated that this mAb is equally potent as omalizumab at blocking IgE-mediated allergic reactions, but does not induce FcγR-dependent adverse reactions. Overall, our data indicate that omalizumab can induce skin inflammation and anaphylaxis by engaging FcγRs, and demonstrate that Fc-engineered versions of the mAb could be used to reduce such adverse reactions.
The anti-IgE mAb omalizumab induces adverse reactions by engaging Fcγ receptors

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Introduction

IgE antibodies (Abs) are key mediators of allergic diseases (1-3). Upon exposure to an allergen in allergic patients, such allergen is recognized by IgE bound to the high-affinity receptor FceRI on the surface of mast cells and basophils, which promotes the immediate activation of these cells and the release of inflammatory mediators such as histamine, responsible for allergic symptoms (3).

Omalizumab (Xolair) is a recombinant humanized IgG1 mAb directed against IgE (4). Omalizumab binds to the Cε3 domain of free IgE, and thereby impairs binding of IgE to both FcεRI and the low-affinity IgE receptor CD23 (FcεRII) (5-7). Omalizumab does not recognize IgE already bound to FcεRI or CD23, and therefore cannot induce cell activation by crosslinking of IgE receptors (5, 7). Omalizumab is approved for the treatment of severe asthma (8) and chronic spontaneous urticaria (9). It also shows promise for the treatment of other allergic diseases, including food allergy (10).

However, treatment with omalizumab is associated with adverse reactions, ranging from skin inflammation at the injection site to anaphylaxis (~0.1%–0.2% of patients) (11-13). The mechanism of these side effects is still unknown. Notably, omalizumab does not induce the formation of anti-drug Abs, and most cases of anaphylaxis occur within the first 3 injections of the drug (11-13).

We hypothesized that the formation of immune complexes (ICs) between omalizumab and IgE could be responsible for some of the adverse reactions observed with this therapeutic mAb. Using mice humanized for all IgG receptors (FcγRs), we demonstrate here that omalizumab/IgE ICs can induce skin inflammation at the site of injection of the drug as well as systemic anaphylaxis through engagement of FcγRs. Finally, we developed an Fc-engineered version of omalizumab that blocks IgE-mediated allergic reactions without inducing FcγR-dependent adverse reactions.

Results and Discussion

We first cocultured omalizumab and human IgE (termed IgE herein) in vitro to form ICs, and assessed the molecular mass of these ICs by size exclusion chromatography coupled to static light scattering (SEC-SLS). As reported previously (14, 15), these ICs were of limited size, mainly consisting of trimeric structures (Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/JCI129697DS1). It was initially suggested that such small ICs have a low potential to engage FcγRs (15). However, we found that these ICs potentially bind all activating human FcγRs (FcγRI, IIA, IIIA, and IIIB), but not the inhibitory FcγRIIB that has the lowest affinity for human IgG1 among FcγRs (16) (Figure 1A). As expected, we also observed that omalizumab binds human complement component C1q in a dose-dependent manner (Figure 1B).
As neutrophils were reported to contribute to IgG-mediated inflammation and anaphylaxis (17), we next evaluated whether omalizumab/IgE ICs can activate neutrophils through engagement of FcγRs. We purified neutrophils from healthy donors and incubated these cells with omalizumab/IgE ICs. We found that such ICs induce marked upregulation of CD66b and downregulation of CD62L on the surface of neutrophils, which are considered hallmarks of neutrophil activation (18, 19) (Figure 1, C and D). The ICs also induced downregulation of FcγRII (CD32) (Figure 1E). As human neutrophils express FcγRIIA and not FcγRIIB (20), and omalizumab/IgE ICs do not bind FcγRIIB (Figure 1A), our results indicate that the ICs induce active engagement of FcγRIIA on neutrophils.

To further confirm the role of FcγRs in neutrophil activation, we performed similar experiments with neutrophils purified from hFcγRII transgenic mice (Figure 1F), demonstrating that omalizumab/IgE can activate neutrophils through engagement of human FcγRs.

The most frequent side effect observed with omalizumab is skin inflammation (13). We hypothesized that such local inflammation could be a consequence of FcγRs engagement. To assess this, we injected omalizumab/IgE ICs subcutaneously into hairless (to avoid shaving-induced skin inflammation) nude hFcγRII transgenic mice and nude FcγRnull mice, and assessed skin inflammation after 2 hours by bioluminescence imaging of myeloperoxidase (MPO) activity (20, 22). We observed strong MPO activity at the site of IC injection in hFcγRII transgenic mice (Figure 2, A and B). By contrast, MPO activity was markedly reduced upon injection of IgE alone or omalizumab alone, or injection of ICs in FcγRnull mice. Thus, our results indicate that omalizumab/IgE ICs can induce skin inflammation through engagement of human FcγRs.

The most dramatic side effect reported for omalizumab is anaphylaxis (12, 13). We thus assessed whether omalizumab/IgE ICs can induce anaphylaxis in hFcγRII transgenic mice. Intravenous...
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The implication of human C1q, our data strongly suggest that the complement pathway plays an important role, through C1q engagement, in omalizumab/IgE-induced anaphylaxis.

Based on these results, we decided to produce an Fc-engineered form of omalizumab (using available omalizumab VH and VL sequences, ref. 4) lacking the N-linked glycan attached to asparagine 297 in the Fc portion (N 297A mutation) to reduce binding to FcγRs and complement (20, 26). We refer to this mAb as NA anti-IgE. As a control, we also produced a nonmutated version of this mAb (WT anti-IgE). Both the WT and NA anti-IgE mainly formed trimers when incubated with IgE in vitro (Supplemental Figure 4, A–D), which is consistent with the data we obtained using commercial omalizumab (Supplemental Figure 1). As expected, ICs made of IgE and the WT anti-IgE could bind all activating FcγRs, whereas binding to FcγRs was markedly reduced with ICs made of IgE and the NA anti-IgE (Figure 3A). Indeed, IgE/NA anti-IgE ICs could only bind to FcγRI, which is consistent with a previous report showing that the N297A mutation does not abrogate binding to this high-affinity FcγR (27). In addition, WT anti-IgE could bind human C1q (Supplemental Figure 4E), but we detected no binding to C1q with the NA anti-IgE (Supplemental Figure 4E). Finally, we observed activation of human neutrophils with ICs made of IgE and the WT anti-IgE, but markedly reduced activation with ICs made of IgE and the NA anti-IgE (Figure 3, B–D).

Injection of ICs induced significant hypothermia (the main readout of anaphylaxis in mice, ref. 23) in hFcγRII mice (Figure 2C). Importantly, hypothermia was not observed upon IC injection in FcγRnull mice (Figure 2C) or injection of IgE or omalizumab alone in hFcγRII mice (Supplemental Figure 2), demonstrating that the ICs induce systemic anaphylaxis through engagement of human FcγRs. Previous work indicates that hFcγRIIA contributes to experimental anaphylaxis in humanized mice (21, 24, 25). We did not observe anaphylaxis in FcγRnull mice transgenic for hFcγRIIA (which express only hFcγRIIA, ref. 21), indicating that hFcγRIIA is not sufficient to trigger omalizumab/IgE-mediated anaphylaxis (Supplemental Figure 3A). By contrast, anaphylaxis was markedly reduced in hFcγRnull mice pretreated with a blocking mAb against hFcγRIII (Supplemental Figure 3B). This suggests that hFcγRIII plays an important role in omalizumab/IgE-mediated anaphylaxis. These results have to be interpreted carefully, as we cannot exclude that pretreatment with the anti-hFcγRIII mAb induces engagement of the receptor to some extent, thereby desensitizing cells expressing hFcγRIII.

Since omalizumab also binds complement component C1q (Figure 1B), we assessed the potential contribution of C1q to IC-induced anaphylaxis. We found that anaphylaxis is markedly reduced in hFcγRIIClq−/− mice, which express all hFcγRs but lack mouse C1q (Figure 2D). Although further work is required to confirm the implication of human C1q, our data strongly suggest that the complement pathway plays an important role, through C1q engagement, in omalizumab/IgE-induced anaphylaxis.

Based on these results, we decided to produce an Fc-engineered form of omalizumab (using available omalizumab VH and VL sequences, ref. 4) lacking the N-linked glycan attached to asparagine 297 in the Fc portion (N 297A mutation) to reduce binding to FcγRs and complement (20, 26). We refer to this mAb as NA anti-IgE. As a control, we also produced a nonmutated version of this mAb (WT anti-IgE). Both the WT and NA anti-IgE mainly formed trimers when incubated with IgE in vitro (Supplemental Figure 4, A–D), which is consistent with the data we obtained using commercial omalizumab (Supplemental Figure 1). As expected, ICs made of IgE and the WT anti-IgE could bind all activating FcγRs, whereas binding to FcγRs was markedly reduced with ICs made of IgE and the NA anti-IgE (Figure 3A). Indeed, IgE/NA anti-IgE ICs could only bind to FcγRI, which is consistent with a previous report showing that the N297A mutation does not abrogate binding to this high-affinity FcγR (27). In addition, WT anti-IgE could bind human C1q (Supplemental Figure 4E), but we detected no binding to C1q with the NA anti-IgE (Supplemental Figure 4E). Finally, we observed activation of human neutrophils with ICs made of IgE and the WT anti-IgE, but markedly reduced activation with ICs made of IgE and the NA anti-IgE (Figure 3, B–D).
We then compared skin inflammation induced by IgE/omalizumab or IgE/NA anti-IgE ICs in hFc\(\gamma\)RKI mice. Injection of IgE/omalizumab ICs induced marked MPO activity in the skin (Figure 4, B and C). This was reduced to levels observed with injection of IgE alone in hFc\(\gamma\)RKI mice injected with IgE/NA anti-IgE ICs (Figure 4, B and C). Finally, we compared the ability of ICs made of IgE and omalizumab or the NA anti-IgE to induce anaphylaxis in hFc\(\gamma\)RKI mice. We observed anaphylaxis in mice injected with IgE/omalizumab ICs but not in mice injected with IgE/NA anti-IgE ICs (Figure 4D).

In summary, our findings demonstrate that omalizumab forms ICs with IgE, which can activate neutrophils and induce skin inflammation and systemic anaphylaxis through human Fc\(\gamma\)Rs in Fc\(\gamma\)-humanized mice. Such findings could explain some of the side effects that have been described in patients treated with omalizumab (12, 13). One must be careful when extrapolating these findings obtained in humanized mice to humans, as very few data have been reported supporting the existence of Fc\(\gamma\)R-mediated anaphylaxis in humans. However, one recent report provides evidence of an IgG-induced, Fc\(\gamma\)R-dependent neutrophil activation pathway in anaphylaxis to neuromuscular-blocking agents (NMBAs) in humans (31), which reinforces the potential clinical relevance of our findings.

The Fc-engineered anti-IgE mAb we used here contains a N297A mutation in the Fc region, which replaces the asparagine residue with an alanine residue (28). We compared the half-life of commercial omalizumab and the Fc-engineered NA anti-IgE (Supplemental Figure 6). We observed similar mAb levels in sera collected at different time points (Figure 3E). Altogether, these results demonstrate that the N297A mutation does not affect the half-life of the anti-IgE mAb in vivo.

We also verified that the N297A mutation does not affect the ability of the anti-IgE mAb to block IgE. Both the WT and NA anti-IgE recognized IgE with the same affinity (Supplemental Figure 7A), and were equally potent at blocking binding of IgE to human mast cells (Supplemental Figure 7B). Moreover, we showed that pretreatment of hFc\(\varepsilon\)RITg mice (which express the human IgE receptor hFc\(\varepsilon\)RI, ref. 30) with either omalizumab or the NA anti-IgE can block IgE-mediated anaphylaxis (Figure 4A). Altogether, our results demonstrate that the Fc-engineered NA anti-IgE is equally potent as omalizumab at blocking IgE-mediated allergic reactions.

We then compared skin inflammation induced by IgE/omalizumab or IgE/NA anti-IgE ICs in hFc\(\gamma\)R\(^{\text{hFcRn/\beta2m}}\) mice. Injection of IgE/omalizumab ICs induced marked MPO activity in the skin (Figure 4, B and C). This was reduced to levels observed with injection of IgE alone in hFc\(\gamma\)R\(^{\text{hFcRn/\beta2m}}\) mice injected with IgE/NA anti-IgE ICs (Figure 4, B and C). Finally, we compared the ability of ICs made of IgE and omalizumab or the NA anti-IgE to induce anaphylaxis in hFc\(\gamma\)R\(^{\text{hFcRn/\beta2m}}\) mice. We observed anaphylaxis in mice injected with IgE/omalizumab ICs but not in mice injected with IgE/NA anti-IgE ICs (Figure 4D).

In summary, our findings demonstrate that omalizumab forms ICs with IgE, which can activate neutrophils and induce skin inflammation and systemic anaphylaxis through human Fc\(\gamma\)Rs in Fc\(\gamma\)-humanized mice. Such findings could explain some of the side effects that have been described in patients treated with omalizumab (12, 13). One must be careful when extrapolating these findings obtained in humanized mice to humans, as very few data have been reported supporting the existence of Fc\(\gamma\)R-mediated anaphylaxis in humans.
developed is equally potent as omalizumab at blocking IgE-mediated allergic reactions but does not induce FcγR-mediated inflammation. It could thus potentially be used in patients with very high levels of IgE and/or in patients with a history of anaphylaxis or other adverse reactions to omalizumab. Finally, we envision that IC-mediated engagement of FcγRs could be a more general mechanism of therapeutic mAb-mediated adverse reactions.

**Methods**

See the Supplemental Methods for the description of all experimental procedures and statistical analyses.

**Study approval.** All animal care and experimentation were conducted in compliance with the guidelines and specific approval of the Animal Ethics Committee CETEA (Institut Pasteur, Paris, France) registered under #2013-0103, and by the French Ministry of Research under agreement 00513.02.

**Author contributions**

BB, PB, and LLR designed the experiments. BB, PH, OG, JS, and LLR conducted experiments. BB, PH, OG, JS, ORL, BI, DS, SB, and LLR acquired data. FMH, VAV, LEM, and AJM provided mice. KCN provided reagents. GAM performed statistical analysis. BB and LLR conducted the formal analysis. BB and LLR wrote the original draft of the manuscript. All authors contributed to review and editing of the manuscript.

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