

Correspondence

Reply

To the Editor:

Our recent studies showing that peripheral blood basophils become more intrinsically sensitive to IgE-mediated stimulation during the treatment of patients with omalizumab¹ raises questions about the mechanisms that could produce this effect. Chirumbolo and Olivieri² have proposed a mechanism that should be considered—one that is an often-ignored aspect of omalizumab treatment. This therapeutic results in immune complexes consisting of IgE and omalizumab, and these complexes may have biological activities that are not recognized. This is, indeed, a potentially important process, but there are several aspects to this issue that warrant consideration.

First would be a consideration of the nature of the immune complexes that form. A couple of studies have suggested that the largest aggregate in serum will consist of 2 anti-IgE antibodies and 2 IgE antibodies that form a cyclized structure.³ Modeling of this reaction suggests that the cyclized dimers or trimers of IgE:anti-IgE are weakly represented during treatment and that smaller aggregates (2 anti-IgE:1 IgE) predominate.⁴ Whether this size aggregate effectively interacts with FcγR on cells is an open question. *In vitro* studies show that allergens can bind to specific IgE in these complexes,⁵ and so it is possible that larger aggregates might be generated in the presence of allergens but this theoretical possibility has not been established *in vivo* or *in vitro*. It is also important to note that despite the immune complex containing an IgE molecule, this IgE molecule is blocked from binding to any unoccupied FcεRI (or FcεRII) so that any interaction with cell-bound receptors would need to occur only with IgG receptors.

A second consideration is whether the concentration of immune complexes that form can compete with the very high concentration of circulating IgG for binding to Fcγ receptors; serum IgG is approximately 50 μM, and omalizumab:IgE complexes are expected to reach maximum concentrations of 1 to 10 nM.⁴ While the avidity of an immune complex considerably exceeds the intrinsic affinity of monomeric IgG for its receptor, the 5000- to 50000-fold difference in concentration between monomeric IgG and immune complexes suggests that complex binding would be minimal.

A third consideration is the type of IgG receptor and in the context of this discussion, the type of IgG receptor on basophils. Although it has been reported that basophils express CD16B (a glycoposphatidylinositol-linked receptor), the expression levels are quite low (10-100-fold lower than other leukocytes) when present and, often, not easily distinguishable from background binding.^{6,7} Furthermore, there is no identified function for the CD16B on basophils when present.^{6,7} This leaves CD32a and CD32b to interact with immune complexes. If the immune complexes can bind effectively to CD32a without interference from CD32b (a possibility for human tissue mast cells), activation might follow and activation could have consequences for signaling molecule expression. Setting aside the involvement of CD32b, it would be expected that Syk participates in a CD32a-mediated activation and if Syk is processed in a manner similar to the processing that occurs during FcεRI-mediated activation, it might be downregulated. However, (1) the omalizumab studies

showed increases in Syk expression,^{1,8,9} not decreases, and (2) one cannot readily set aside the role of CD32b which is predominately expressed on basophils and is functionally dominant as well.^{7,10} With CD32b involved, signaling may be suppressed with an unknown consequence on the speculated loss of Syk with CD32a activation. One might guess that Syk would continue to be downregulated even if CD32b was involved because this is the behavior during combined FcεRI and CD32b activation¹⁰ but this would explain only a loss of Syk rather than increased expression. At this point in time, there is no known mechanism that involves IgG receptors, even if they could be activated, that would explain the increase in intrinsic sensitivity to FcεRI-mediated stimulation.

Chirumbolo and Olivieri made a few assertions that should be addressed. Notably, that (1) clearance of immune complexes leads to loss of FcεRI on basophils, (2) omalizumab can “strip” bound IgE from FcεRI, (3) IgG receptors can modulate FcεRI expression, (4) there is competition for FcRβ between IgE and IgG receptors, and (5) CD32b activation leads to lyn kinase activation/phosphorylation.

First, there is no experimental evidence that immune complex clearance leads to the downregulation of FcεRI (immune complexes binding to allergen might stoichiometrically reduce free allergen binding to cells, but circulating allergen-specific IgG can do this). Notably, receptor expression is downregulated by the simple absence of IgE because of the characteristics of this receptor's cycling and production.^{11,12} Second, and most importantly, omalizumab does not induce the dissociation of IgE from FcεRI. This has been studied extensively in published and unpublished studies.^{13,14} The citation provided for the assertion in point 2 cannot be evaluated because it is a meeting abstract, but it runs counter to published studies. There are reports of other anti-IgE antibodies capable of inducing dissociation and it is theoretically possible, but apparently not for omalizumab. Third, there is no evidence, including those citations provided, that IgG receptors can modulate FcεRI expression (rather than function). Fourth, while it is possible that there is competition between FcεRI and IgG receptors for a shared beta subunit, the relative levels of expression of the 2 classes of receptors and the relative abundance of the beta subunit⁸ suggest that this is not a meaningful characteristic of the system. In addition, the observations also suggest that in human basophils, the beta subunit does not act as an amplifier. Studies with omalizumab have shown that when FcεRI expression is reduced during treatment, there is a greater and disproportionate downregulation of FcRβ⁸ while at the same time the cell is becoming more sensitive to stimulation.¹ While there are multiple explanations for this discordance that preserve the role of beta as an amplifier, taken at face value, this set of results does not support its role as an amplifier. Fifth, the studies cited refer to CD64 mediating lyn phosphorylation, not CD32b.

In our early studies of omalizumab treatment and its effects on FcεRI expression, we considered the potential role of immune complexes in mediating some of the biology observed. There may be good reasons to consider this possibility but to this date, there has been no evidence to support this possibility and some of the theoretical considerations make it difficult to imagine how such modulation might occur.

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