Reply

To the Editor:

We thank the authors for their well-thought-out comments 1 on our study. 2 As they have stated, the 300-mg subcutaneous mepolizumab dose investigated in the Mepolizumab In Relapsing or Refractory EGPA (MIRRA) study was chosen to encompass a broad population and was informed by modeling and simulation. Compared with severe asthma, eosinophilic granulomatosis with polyangitis (EGPA) involves greater implication of eosinophils at multiple target organs, and there is potential for a significant increase in blood eosinophil counts preceding relapse or during glucocorticoid taper. Therefore it was considered that a higher dose of mepolizumab would be required in patients with EGPA to confer therapeutic benefit compared with severe asthma.

To enter the MIRRA study, patients were required to have received a diagnosis of EGPA, including a history of hypereosinophilia (blood eosinophil count >1000 cells/µL and/or >10% blood leukocytes). All patients were also required to receive a stable dose of 7.5 mg/d or greater of prednisone/prednisolone (but not >50 mg/d) at baseline, which, along with optional immunosuppressive therapy, would affect blood eosinophil counts. Because physicians were permitted to taper glucocorticoids throughout the study, the 300-mg subcutaneous dose was selected to manage a spike in blood eosinophil counts that can occur after withdrawal of glucocorticoid treatment. This translates well into clinical practice because a major objective of physicians treating EGPA is to decrease glucocorticoid/immunosuppressive treatment.

Although broad efficacy in patients with EGPA was demonstrated with 300 mg of subcutaneous mepolizumab, it is possible that for some patients a higher dose might improve outcomes to an even greater extent or that lower doses might prove to be just as effective. We acknowledge that it is plausible that additional research could identify patients with EGPA who might benefit from mepolizumab treatment and lead to personalized dosing regimens matched to disease progression and severity. Nonetheless, based on available data, we recommend the approved dose of 300 mg of subcutaneous mepolizumab every 4 weeks for treatment of adult patients with EGPA.

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Basophil histamine release in patients with chronic spontaneous urticaria: Optimize or minimize

To the Editor:

A recent article by Dr MacGlashan 1 concludes that the “true” incidence of IgE receptor antibody in patients with chronic spontaneous urticaria is less than 10%. 4 Although most studies report an incidence of 25% to 45% positive basophil histamine release, constraints were added to the analysis, including dependence of basophil histamine release of SYK, Bruton tyrosine kinase, and phosphoinositide 3-kinase, which are linked to IgE receptor activation, plus successful desensitization using aggregated IgE. The initial histamine release value of 41% was then decreased to 7%.

There are at least 2 confounding issues with this analysis. Serum levels of purified IgG from patients’ sera yields 2- to 3-fold less histamine release compared with whole serum because of complement activation during the course of the reaction with release of C5a. C5a will not fulfill the constraining criteria, yet the complement activation is IgG dependent and is prevented when pathogenic IgG is added to C2- or C5-deficient serum. The process was inhabitable by incubation with the α subunit of the IgE receptor, 2,4,5 IgG-depleted sera were negative, and addition of myeloma IgE to saturate IgE receptors sterically blocks histamine release by anti-FcεRIα.

Then there is an issue of sensitivity. Sera were used at a 1:4 dilution or less, whereas we and others assay sera undiluted or at 1:2 dilution. Our incidence of 40% to 45% uses basophils of 2 particular subjects chosen for high releasability and low stable baseline values. Values beyond 15% release were considered positive to avoid potential confounding effects of serum enzymes, histamine in serum (chronic urticaria), bradykinin in serum positive to avoid potential confounding effects of serum enzymes, baseline values. Values beyond 15% release were considered positive to avoid potential confounding effects of serum enzymes, histamine in serum (chronic urticaria), bradykinin in serum (produced by clotting), and histamine-releasing cytokines. Predilution of sera plus dilution with reagents might substantially lower the percentage of positive values, even when controlled internally.

Assessment of the activity in sera has gone beyond phenomenology. Histamine-releasing activity was predominantly IgG2 and IgG3, which activate complement. No positive result has been obtained with IgG2 (but one was IgG4), 5 and IgA, positive binding to the IgE receptor by immunoblotting, does not cause histamine release when purified. Thus binding assays are unreliable 5,6 as a predictor of function.

The study by MacGlashan 1 further dissects one aspect of the problem, namely the features guiding basophil responsiveness and criteria to assume action through the IgE receptor. This approach is an important addition. However, the studies referenced above are also the only ones that have analyzed serum determinants behind the observed basophil activation. Both approaches are needed to best delineate the role of autoimmune phenomena in patients with chronic spontaneous urticaria, even though cutaneous mast cells would be preferable. Thus although

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the incidence of histamine-releasing sera could vary from a low of 7% to a high of 45% (or zero using “nonreleasing” basophils), it is my opinion that the histamine release ought to be optimized and not minimized. Specificity might be diminished; however, in our laboratory this has been minimal, and it is well known that most autoantibodies have a low percentage of positive results in healthy subjects.

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Reply

To the Editor:

Dr Kaplan has raised some valid concerns about the methodological details that are used to assess functional autoantibodies in patients with chronic spontaneous urticarial (CSU). Although the study goals in my report were distinct from attempting to enumerate the frequency of functional autoantibodies in the population with CSU, it was a byproduct of the study that obtained an estimate of this frequency.

Dr Kaplan is correct to note that my study’s use of 25% serum placed a partial limit on sensitivity because it was possible that either undiluted serum was necessary to observe some function or that additional components, such as complement, were needed to drive or augment the reaction. I would also note that the study used a threshold for positivity that might have missed serum-induced secretion that was very low but consistent. The threshold of 6% was chosen after analyzing the amount of noise across all the pilot assays that had been done for the study, but because the purpose of the study was to understand whether functional autoantibodies (that could be demonstrated to operate through FcεRI) altered SYK expression, the threshold of 6% ensured that subsequent tests could be interpreted. However, as noted, a somewhat lower threshold might have allowed additional serum to be included as positive. This issue is relevant to the design of a consistent approach to the problem of characterizing serum to be discussed further below.

That said, there are other interesting byproducts of the study. Notably, not all release induced by serum appears attributable to signaling activity through the high-affinity IgE receptor. From my perspective, this was revealed most starkly in the experiments presented in the online supplement, showing that a simple overnight incubation with IL-3 generated basophil preparations that could respond to all serum (ie, regardless of source). The induced histamine release was not inhibited with potent agents that operate on FcεRI-mediated signaling. This result simply magnifies the potential for misinterpretation of the results when using serum. IL-3 is known to augment the response to all basophil secretagogues, but the uniformity of the result across all sera points to a problem in working with this assay. The study did not deeply explore this phenomenon. In particular, it did not test whether IL-3 could cause problems after only short exposures (minutes). This would be important to know because many basophil activation assays today incorporate IL-3 into the reaction buffers (which, by the way, they should not for mechanistic research studies).

The basophil does express a very active C5a receptor and a C3a receptor that generates a weak reaction, and therefore an autoantibody-binding reaction that is ultimately dependent on complement might depend on the nature of the serum and its concentration. This aspect of autoantibody functionality would certainly not be counted in my study, although it was not relevant to the goals of the study (eg, C5a receptor activation is not known to modulate SYK expression). Nevertheless, an experimental demonstration that complement activation is a requirement for a Basophil Activation Test of autoantibodies is also not a well-established component of assays that report positivity, and therefore it is unclear how to assess this possibility.

I would agree with Dr Kaplan that an important issue raised by my study is whether it is possible to (1) develop a completely consistent methodology useful to all investigators for assessing the presence of autoantibodies and (2) develop a way to assess not only directly functional autoantibodies but also antibodies that either bind too poorly to be detected by using this type of assay (but that might have long-term effects in vivo) or antibodies that bind but use alternative pathways to activation (eg, complement). We are currently working with a basophil assay considerably simplified from the previous report. We incorporate a Bruton tyrosine kinase inhibitor in the reaction and for further testing do the same after dissociating some IgE from the cell surface to capture the effects of autoantibodies that are sensitive to the occupancy of FcεRI (as some appear to be). However, the issue of what would constitute a consistent assay for all investigators is complex. For example, as noted above, the issue of what constitutes a positive signal seems as if it should be simple to define, but investigators use different methods to assess the basophil response to serum, and each of the approaches have specific issues that change the threshold. One laboratory’s 15% response might be statistically justified but similar to a 6% response using a different assay protocol and measurement methodology (eg, histamine release by means of ELISA vs an autoanalyzer vs flow cytometry). A flow cytometric Basophil Activation Test cannot test 100% serum. The many differences in study results across the literature exploring this issue, including some studies not able to discriminate between disease states and nondisease states, suggests that a more refined approach is needed.