

Correspondence

Reply

To the Editor:

Dr Kaplan¹ 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 report² 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 Kaplan¹ 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 expression³). 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 Kaplan¹ 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 reaction⁴ 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,⁵ suggests that a more refined approach is needed.

It could be argued that my study was optimizing the assay to exclusively detect directly functional antibodies. If there are other mechanisms causing basophil responses, they too should be independently enumerated to better understand the relationship of serum endotype to the expression of active CSU.

As Dr Kaplan¹ noted, there is considerable knowledge today about secretion from basophils and some selective tools for helping to define the nature of the reaction, and my own takeaway from the study has been that we should be using some of these tools. The wide variety of issues and the results in my report suggest that the commercial assay for antibodies might need some reconsideration.

Donald MacGlashan, Jr, MD, PhD

From the Division of Allergy and Clinical Immunology, Johns Hopkins University, Baltimore, Md. E-mail: dmacglas@jhmi.edu.

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