

Complexities in analyzing human basophil responses to autoantibodies to IgE or FcεRI



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Secretion of cytoplasmic granules (ie, degranulation) and production of other mediators not stored in granules (eg, leukotrienes, prostaglandins, and diverse cytokines) by mast cells and basophils activated through FcεRI play key roles in diverse IgE-mediated immune responses, including asthma, hay fever, food allergies, and anaphylaxis.^{1,2} In both mast cells and basophils, secretory granule structure is thought to reflect in part electrostatic complexes between proteases (positively charged) and proteoglycans (negatively charged), which associate with and thereby help store multiple bioactive molecules, including histamine and certain cytokines.^{1,2} Even though basophils usually represent less than 1% of circulating peripheral blood leukocytes, analysis of basophil activation (ie, using basophil activation tests) has become popular,³⁻⁵ both because basophils can play specific roles during type 2 immune responses and allergic disorders and because blood basophils are much more readily available for analysis than tissue-resident mast cells.

However, it is thought that mast cells and basophils can also be activated by autoantibodies to IgE, FcεRI, or both. As reviewed by MacGlashan,⁶ several studies have analyzed the potential roles of autoantibodies to IgE, FcεRI, or both in eliciting important roles for basophil activation in patients who might not have classical allergic disorders, notably those with chronic spontaneous urticaria (CSU). In a carefully designed and rigorously performed study, MacGlashan⁶ examined how autoantibodies against FcεRI, which are more frequently found in patients with CSU than in unaffected subjects, relate to levels of the downstream signaling molecule spleen tyrosine kinase (SYK). The author hypothesized that if the autoantibodies identified in such patients were functional antibodies, variability in their activity might explain variability in SYK expression in basophils, which in turn has been linked to clinical responsiveness to therapy in patients with chronic urticaria, food allergy, and anaphylaxis.

Surprisingly, through carefully controlled experiments, MacGlashan⁶ concluded that in most (but not all) cases the autoantibodies of patients with CSU lacked the capacity to activate donor basophils reproducibly. These findings support prior work indicating that many anti-FcεRI antibodies in patients with CSU are nonactivating when tested against donor basophils.^{7,8} MacGlashan⁶ also was unable to detect a relationship at baseline between the presence of autoantibodies, whether functional or nonfunctional, and levels of SYK expression in basophils.⁶

Although the finding that most of the anti-FcεRI antibodies in patients with CSU are nonactivating for basophils is important and might explain some of the controversies in the field⁶; this finding does not necessarily rule out the ability of such autoantibodies to activate tissue mast cells. Niimi et al⁹ found that circulating levels of anti-FcεRI autoantibodies from 12 selected patients with severe chronic idiopathic urticaria, whose sera could release histamine from donor basophils, were also able to mediate histamine release from pieces of human foreskin *in vitro*. Unfortunately, skin-resident mast cells are difficult to isolate, and work with such cells would help discriminate direct from indirect effects of anti-FcεRI autoantibodies on mast cells. Moreover, experiments using cord blood- or CD34⁺-derived mast cells (which do not share all of the functional characteristics of tissue-resident mast cells) can yield results that depend importantly on the functional properties of mast cells generated *in vitro* but might not apply to those mast cells found in real environments occupied *in vivo*.

An important second point is that the study by MacGlashan⁶ was not initially designed to investigate the proportion of sera from patients with CSU that could elicit an FcεRI-dependent response. Instead, his goal was to find natural autoantibodies that could be used to modulate SYK expression in basophils and to assess whether such autoantibodies detectably interacted with FcγRIIb/CD32b as part of their ability to influence SYK expression. As noted by the author,⁶ there has been documentation of extensive heterogeneity in basophil SYK expression, as reflected in the broad distribution of maximal IgE-determined histamine release (eg, Puan et al¹⁰). However, the absence of natural autoantibodies in subjects without CSU shifted the author's attention to patients with CSU, and the current article is the result of this redirection of effort.

The thorough technical development of the author's experimental algorithm for classifying the nature of histamine release induced by sera from the 3 groups of subjects analyzed is notable. MacGlashan⁶ studied sera from (1) subjects without CSU "whose basophils were well characterized for their releasing properties, including IgE-mediated histamine release,"⁷ as well as sera from patients with CSU followed at Johns Hopkins and well characterized clinically regarding the expression of CSU, including (2) specimens that were collected independently of the presence of basopenia at phlebotomy and (3) those from a second request from patients with CSU known to have basopenia. As detailed in the article,⁶ the thresholds for "positivity" of basophil

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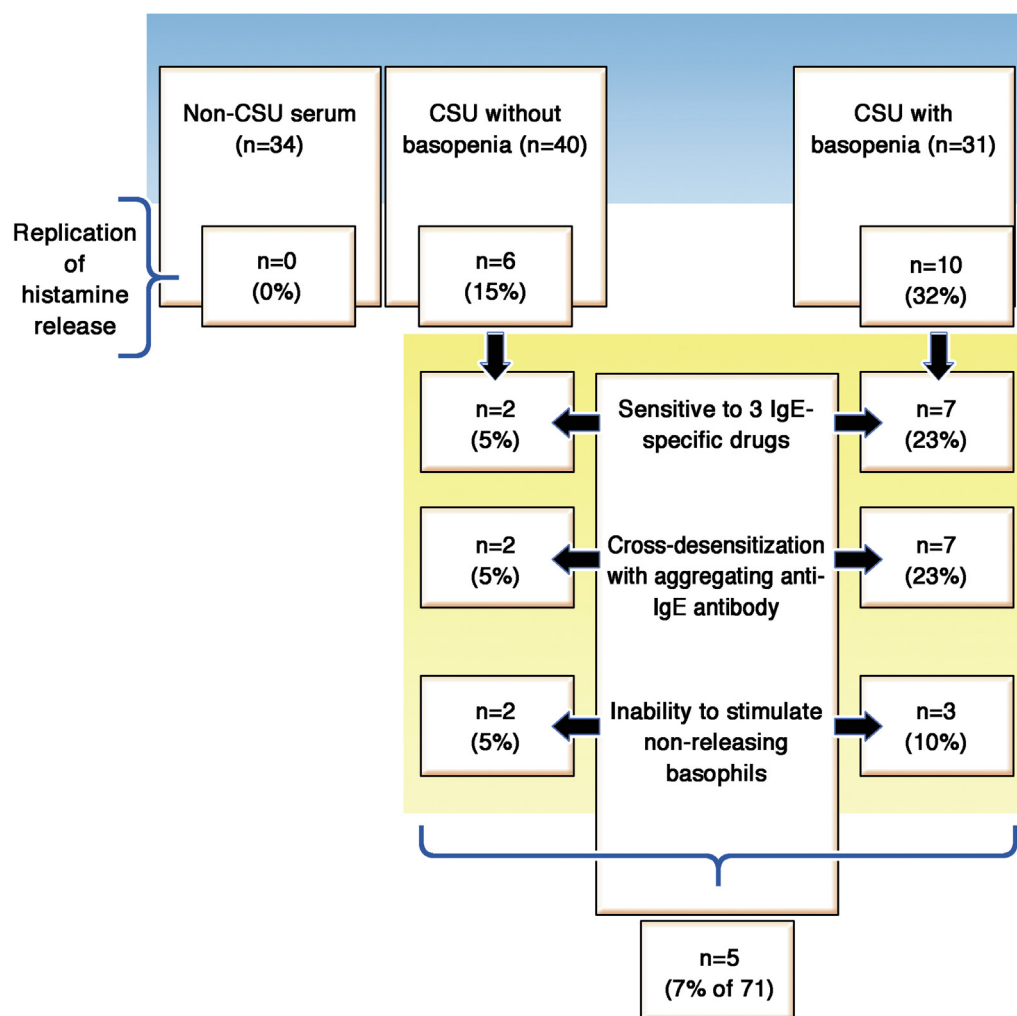


FIG 1. Criteria for identification of sera that contained functional autoantibodies to FcεRI or IgE. Note that the basophils tested were from donors whose basophils were known to be sensitive to stimulation through either FcεRI or IgE. Also, replication of histamine release was shown by the results obtained when 1 serum was tested with basophils of 2 distinct donors. Sera from subjects without CSU were derived from both allergic (25%) and nonallergic (75%) subjects. This figure is a modification of Fig 1 in MacGlashan.⁶

degranulation might have been addressed in a number of ways (and various thresholds have been used by others studying this phenomenon), but the one chosen took histamine release of at least 6% (above spontaneous release) as indicative of “positivity.” Moreover, in his study MacGlashan⁶ used an upper limit of 25% serum in assessing samples, reflecting the needs of the autoanalyzer for the histamine used. Indeed, by the end of this study, the criteria for a serum to be classified as containing functionally active antibodies to IgE or FcεRI also included that the serum could be depleted of its activity by a solid matrix containing IgE or FcεRIα and that the serum would induce a loss of SYK expression during overnight basophil culture.⁶

According to these stringent criteria, MacGlashan⁶ found that the frequency of functional autoantibodies that could produce characteristics concordant with FcεRI-mediated secretion was zero in 34 subjects without CSU (importantly, these included some subjects whose basophils had nearly no histamine release and therefore little SYK expression; Fig 1). Surprisingly, according to MacGlashan’s criteria, the frequency of such functional

autoantibodies in patients with CSU was substantially lower than expected at approximately 7% (Fig 1).⁶ For the 5 of 68 unique sera from patients with CSU tested that contained anti-FcεRI or anti-IgE antibodies, these antibodies induced downregulation of SYK in both peripheral blood basophils and basophils developed from CD34⁺ progenitors. Notably, however, blocking the interaction of these antibodies with FcγRIIb/CD32b did not alter their ability to downregulate SYK expression,⁶ providing additional evidence that certain autoantibodies can induce changes in SYK expression without encountering substantial interference caused by binding of their Fc region to FcγRIIb/CD32b. Finally, although the results were few and presented cautiously, in the 3 instances in which “positive” sera were tested with sera obtained a year or more later, the positive results were not repeated.

The author concluded that the presence of functional autoantibodies to IgE, FcεRI, or both does not provide a good explanation for the high variability in SYK expression in basophils in the general population, 99% of which do not express CSU. On the other hand, the results showed that when antibodies

with these characteristics are present (although in <10% of patients with CSU, at least according to MacGlashan's stringent criteria), then they are capable of modulating SYK expression in developing basophils, although apparently without significant interaction with Fc γ RIIb/CD23b.

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