

IgE to galactose- α -1,3-galactose and the α -Gal syndrome: Insights from basophil activation testing



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Since the earliest reports describing a connection between the galactose- α -1,3-galactose (α -Gal) syndrome and tick bites, it has been clear that the prevalence of IgE sensitization to α -Gal in some regions and select populations is 10% or greater, with population estimates of approximately 20% in studies from Virginia, North Carolina, and Tennessee.¹⁻³ On the other hand, the question of the prevalence of cases of *bona fide* α -Gal syndrome (ie, subjects who manifest allergic symptoms on ingestion of mammalian meat or innards) is less clear and has not been investigated in the United States.

The best evidence comes from the work of Fischer et al,³ who assessed a high-risk population of 300 hunters and forest workers in southwest Germany and determined that only 8.6% of those who were sensitized (at an α -Gal-specific IgE [sIgE] cutoff of ≥ 0.35 IU/mL) had clinical histories or allergic reactions to mammalian meat. Although this finding warrants additional investigation, it suggests the possibility that the majority of subjects with detectable IgE to α -Gal are not clinically “allergic.” This is not different from other food allergies because practicing clinicians are often faced with the dilemma of making sense of positive sIgE blood test results with a negative or indeterminate clinical history. Further complicating matters, because the α -Gal syndrome has a characteristic delay between ingestion and symptom onset of 3 to 6 hours, conducting oral food challenges with red meat in the clinic presents significant challenges (and potentially risks).

On this backdrop Mehlich et al⁴ set out to determine whether the basophil activation test (BAT) could provide a useful tool to discriminate between subjects who had α -Gal syndrome ($n = 21$) and those who were sensitized to α -Gal but tolerated mammalian products ($n = 12$). As previously described, basophil activation reflects a number of biologic variables that are not necessarily accounted for by serum IgE testing.⁵ The approach taken in the present report was to isolate and

stimulate PBMCs with a panel of different α -Gal-containing proteins or foods (eg, pork kidney) and to assess by means of flow cytometry the percentage of basophils that were positive for the surface activation marker CD63 (positive cutoff, $>15\%$; Fig 1).^{4,6} An additional metric included a ratio calculated based on the percentage of basophils activated with α -Gal exposure compared with the percentage activated by anti-Fc ϵ RI antibody treatment. Several test conditions performed well for discriminating between the 2 populations and achieved an area under the curve (AUC) in receiver operating characteristic analysis of 0.95, which exceeded the AUC of 0.78 for α -Gal sIgE testing. The authors conclude that the BAT could be a tool for clinicians to stratify allergy risk and inform decision making among α -Gal-sensitized patients with an indeterminate history.

The BAT could indeed hold promise in the future, although there are several caveats worth considering. In contrast to skin testing or serum sIgE testing, the BAT is not readily available to most practicing allergists. Moreover, implementing BATs outside of research settings also introduces questions about reproducibility and cost that have not been satisfactorily addressed to date. In addition, it is not clear that the performance characteristics of the BAT in this cohort, which only included 26 subjects in the final analysis (of 33 enrolled), are sufficiently superior to warrant their implementation in a clinical setting. Despite the high AUC reported for the BAT, it seems unlikely there is a single cut point that is both highly sensitive and highly specific for predicting which subjects sensitized to α -Gal are clinically allergic (a feature that is shared by skin testing and serum IgE testing). For example, results of pork kidney stimulation at 0.227 ng/mL, which was one of the optimal BAT conditions (AUC = 0.95), were positive in 57% of subjects with α -Gal syndrome and 0% of the sensitized group (using 15% basophil positivity as the cut point). By contrast, results of intracutaneous skin testing with a commercial gelatin preparation (4% Gelafundin [B. Braun; Melsungen AG, Melsungen, Germany]) were positive in 93% of subjects with α -Gal syndrome but only 13% of the sensitized but asymptomatic subjects. Thus the intracutaneous skin test had greater sensitivity but only a modest loss in specificity compared with the BAT. A similar result to that seen with intracutaneous testing was revealed with serum sIgE testing by calculating the percentage of α -Gal sIgE in relation to total IgE using a cutoff of 3% α -Gal sIgE (90% vs 17%). Nonetheless, a real strength of the work by Mehlich et al⁴ is the head-to-head comparison of different testing modalities in a well-defined patient population.

A further caveat to consider relates to the possibility that IgE sensitization to α -Gal could have health risks that extend beyond anaphylaxis. This stems from a recent report demonstrating an association between sIgE to α -Gal and coronary artery disease.⁷

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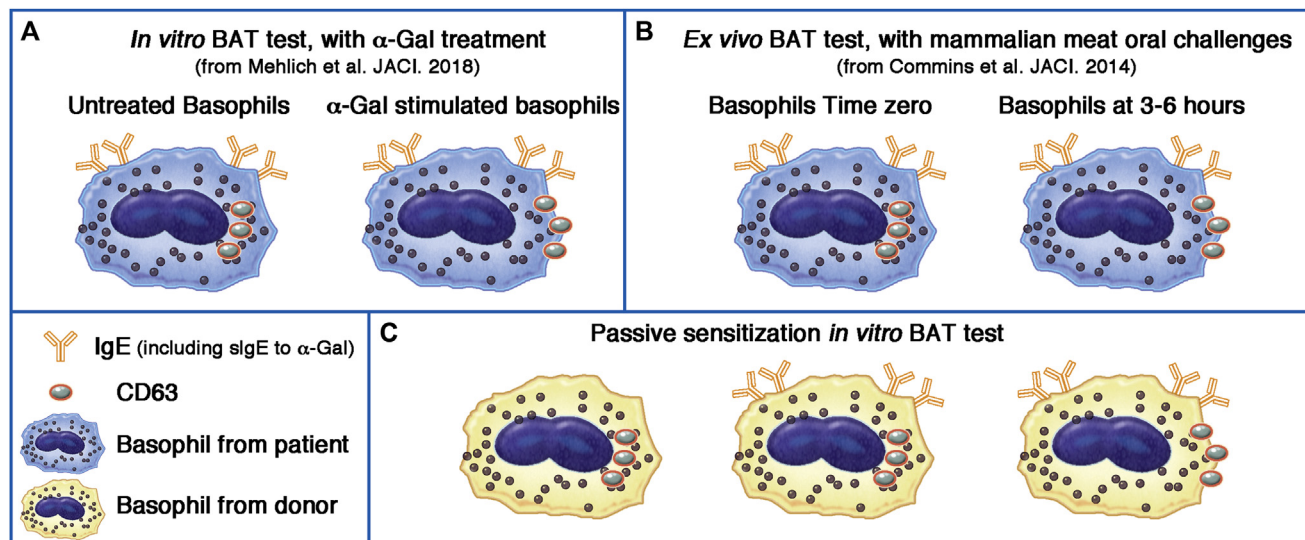


FIG 1. Three forms of BATs. **A**, The current report demonstrated that basophil activation can occur rapidly with *in vitro* stimulation by α -Gal-bearing foods or proteins. Basophil activation was greater in subjects with α -Gal syndrome than in those who were sensitized but tolerated mammalian products. Importantly, baseline levels of basophil activation, as assessed by means of cell-surface expression of CD63, were also greater in subjects with α -Gal syndrome. **B**, The *ex vivo* test demonstrated that basophil activation in patients with α -Gal syndrome occurs after challenge, with similar kinetics as the characteristic delay of 3 to 6 hours that is observed clinically. **C**, Possible experiment using passively sensitized basophils. In this approach basophils from an unrelated donor are isolated and stripped of surface-bound antibodies by using an acid-treatment step, and serum from a patient is incubated and allowed to bind to the basophils. Compared with model A, this approach would provide information about the role of serum constituents (ie, IgE and IgG antibodies) versus the intrinsic properties of allergic effector cells (ie, basophils) in determining clinical reactivity in subjects who are sensitized to α -Gal.^{4,6}

Although the cohort was small and there are currently no mechanistic studies to support the observation, the association is biologically plausible. A working model to explain the connection proposes that chronic ingestion of mammalian products in subjects who are sensitized to α -Gal could contribute to persistent low-grade inflammation in the walls of the coronary arteries. Although data are currently insufficient to make recommendations for changing clinical practice, this study has given us pause in thinking about the advice we give to our α -Gal-sensitized patients about diet, particularly those that have established risk factors for heart disease.

Beyond the possible clinical merits of the BAT, perhaps the greatest contribution of the current report relates to suggested insights into α -Gal biology. Previously, it was proposed that differences in gut microbiota, gut barrier function, or systemic metabolism could explain why some subjects who are sensitized to α -Gal react clinically to mammalian products but others do not.⁸ Although the current work does not preclude these possibilities, it is interesting that patients with α -Gal syndrome had significantly higher tryptase levels, as well as baseline basophil activation. This suggests that some of the differences between responders and nonresponders could be accounted for by differences in the intrinsic activation of allergic effector cells (ie, mast cells and basophils), a possibility that would be consistent with the recent report by Carter et al.⁹ It is also important not to discount the effects that sIgE titer or affinity could be playing. After all, patients with α -Gal syndrome had significantly greater titers on serum testing in this cohort. Conducting the BAT with a passive sensitization technique could be another way to approach this. It is also important to recall that an *ex vivo* BAT study has

already provided important mechanistic evidence that supports the characteristic delay of 3 to 6 hours that is observed clinically (Fig 1).⁶

Taken together, although the BAT has the potential to be a useful tool in clinical practice, to date, the BAT has played a more important role in addressing questions regarding the immune mechanisms that contribute to hypersensitivity reactions. This has been particularly true in the case of α -Gal syndrome, a food allergy with several unusual features. It is clear that the BAT and also mast cell activation testing will provide additional insights in the future.¹⁰

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