Letter to the Editor

Dose-time-response relationship in peanut allergy using a human model of passive cutaneous anaphylaxis

To the Editor:

The extensive use of the double-blind, placebo-controlled oral food challenge in the diagnosis of food allergy and the growing uncertainties about the appropriate time interval between dose steps highlight the need to better understand which factors govern the reaction time in anaphylaxis, that is, the time it takes for an allergic reaction to develop after a patient has ingested the allergen.[1] The purpose of this study was to determine whether the serum level of allergen specific IgE (sIgE) and/or the oral challenge dose affect the reaction time (T_{react}) and the size of the wheal (S_{wheal}) in IgE-mediated, cutaneous reactions *in vivo*.

Details of the methods can be found in this article's Methods section in the Online Repository at www.jacionline.org. Briefly, we used the method of passive cutaneous specific hypersensitivity to test our hypotheses in a patient-safe manner. In this technique, which Prausnitz and Küstner were the first to describe,[2] human serum from 4 donors with severe peanut allergy was injected intradermally into nonallergic individuals (the recipients, 41 healthy adults). The sIgE levels to peanut/Ara h2 of the donor sera were 7.5/1.7, 60.1/36.3, 61.8/45.0, and 93.1/71.0 kIU/L (see Table E1 in this article's Online Repository at www.jacionline.org). After the serum injections, the recipients underwent oral challenges with the causal allergen (peanut) to elicit an immediate and local allergic skin reaction at the original sites of the serum injections. The oral challenges were performed either as single dose or in a titrated protocol.

In this study, T_{react} was defined as the time from the challenge dose was swallowed to the first signs of erythema appeared at the sensitized skin site, but it was considered positive only if a wheal also subsequently developed. S_{wheal} was measured when the wheal was at its maximal size.

All donors met the specific eligibility criteria outlined by the Danish Blood Safety Directives.[3] The Regional Scientific Ethics Committee for Southern Denmark approved the study protocol (Project-ID: S-20130086).

The median T_{react} decreased when the serum from the same donor was less diluted, that is, when the sIgE levels to peanut and Ara h2 increased (solid lines in Fig 1). All recipients reacted at the skin sites sensitized with the 3 highest sIgE levels, whereas only 4 of 10 and 7 of 10 reacted to the 2 lowest sIgE levels (gray bars in Fig 1). The importance of the level of sIgE was further substantiated by comparing the sera from the 4 donors with different sIgE levels (see Fig E1 in this article's Online Repository at www.jacionline.org). In addition, the median T_{react} of the serum with the lowest sIgE to peanut (donor 1; see Table E1) and the 10% serum from the dilution experiment (Fig 1) were comparable.

The median T_{react} also decreased with increasing oral doses of peanut in the single-dose challenges (Fig 2, *A*). The pattern observed for S_{wheal} was an increasing wheal diameter with increasing doses of peanut (Fig 2, *B*). Finally, the titrated oral peanut challenges with 1-hour dose intervals demonstrated large variations in threshold mimicking the real-life situation in patients (see Fig E2 in this article's Online Repository at www.jacionline.org).

This study is the first to investigate the significance of the level of allergen sIgE on the reaction time (T_{react}) in the allergic response as well as the significance of the challenge dose in relation to T_{react} and the size of the resulting wheal (S_{wheal}) of IgE-mediated cutaneous reactions using a human model of passive cutaneous anaphylaxis. We found that an increase in the sIgE level as well as an increase in the challenge dose shorten T_{react} in the allergic response. We also found that a higher challenge dose increases the magnitude of IgE-mediated cutaneous reactions, that is, S_{wheal}. Our findings are in keeping with those of Brunner and Walzer[4] as well as Aoki et al.[5] Both studies showed that a wheal-and-flare reaction first appears at the skin site sensitized with undiluted serum and then later at the sites of decreasing serum concentrations.

The results from the challenges in our study indicate that a dose-time-response relationship applies for IgE-mediated reactions: a short T_{react} correlates with a high sIgE level and a high challenge dose, and a small S_{wheal} correlates with a low challenge dose. However, T_{react} appeared to reach a maximum at a certain sIgE level seeing that there was no significant difference in T_{react} for the sera with Ara h2 levels from 36.3 kIU/L to 71.0 kIU/L (Fig E1). Similarly, a maximum response, that is, S_{wheal}, appeared to be reached at 10 g of peanut in our recipients (Fig 2, *B*).

This study also showed that the sIgE level had to be above a certain level for all recipients to respond to the same dose of peanut (Fig 1 and Fig E1) and at the lowest challenge doses several recipients did not react even though they had been sensitized with equal amounts of sIgE from the same donor (Fig E2). This between-subject biological variation could be explained by differences in the gastrointestinal absorption of peanut and that a certain serum concentration of the allergen is required for the reaction to develop given that neither body weight nor body mass index correlated with the response (data not shown).

The results mimic classical pharmacokinetics and pharmacodynamics, and perhaps studies on allergen uptake, distribution,

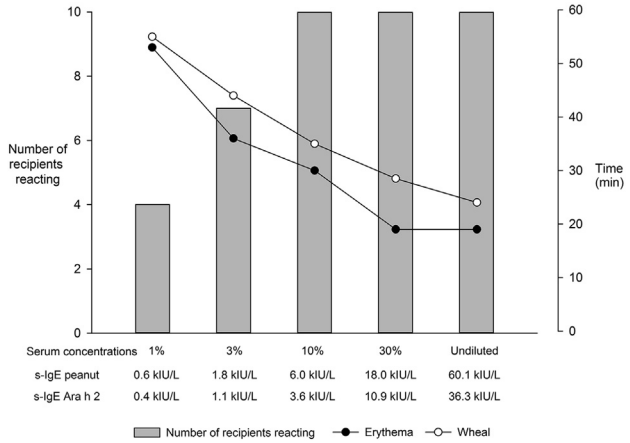

FIG 1. The number of reacting recipients (gray bars) and the median T_{react} (●/erythema and o/wheal) in relation to the level of sIgE. The study includes 10 recipients sensitized with serum from donor 2 (Table E1) in 5 increasing concentrations. The oral peanut challenges were carried out as single dose (10 g of whole peanut).

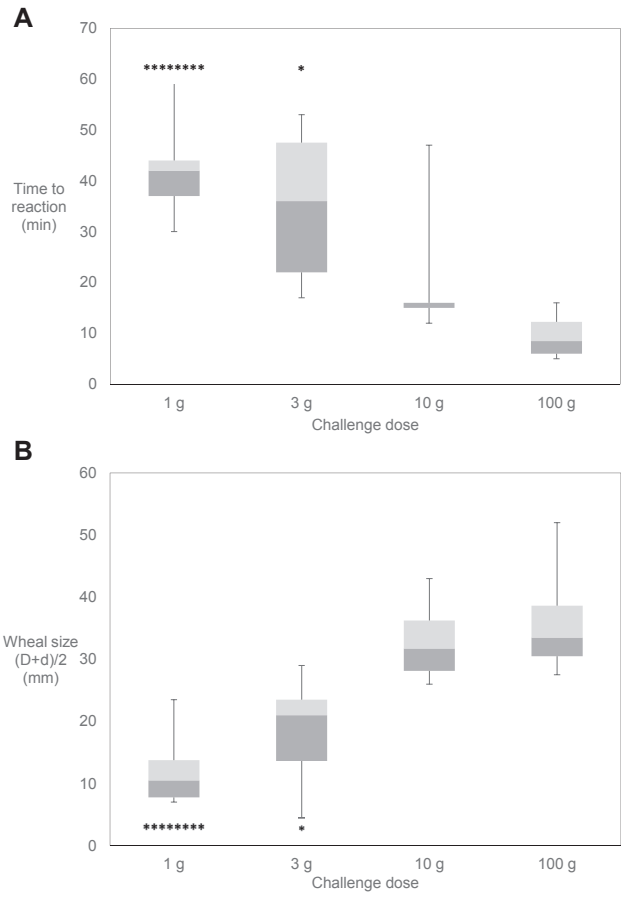

FIG 2. **A,** The median T_{react} in relation to the challenge dose. **B,** The median S_{wheal} in relation to the challenge dose. Both graphs (box plots) display the distribution of the data as follows: the median, the 25th and 75th percentiles (the boundary of the box), and the highest and lowest values (the whiskers above and below the box). All recipients were sensitized with donor serum 2, 3, and 4 (Table E1). The difference in the median T_{react} between all doses compared pairwise was statistically significant ($P \leq .003$) except for the difference between 1 g and 3 g of peanut ($P = .4$). The difference in the median S_{wheal} between the doses compared pairwise was statistically significant ($P \leq .001$) except for the differences between 1 g and 3 g of peanut ($P = .05$) and 10 g and 100 g of peanut ($P = .1$). The *asterisks* indicate that a reaction was negative.

and excretion thus should be interpreted in an identical fashion. This has already been demonstrated in an animal model.[6] However, to thoroughly investigate the kinetics, it would be necessary to identify and measure the absorbed (and as yet unknown) allergen(s) in the blood and then compare the plasma concentrations to *in vitro* histamine release from basophils or mast cells. Although all recipients demonstrated a positive skin prick test result to a crude peanut extract at the sites of the serum injections, an *in vitro* dose titration using basophils or mast cells would make little sense due to the lack of data on the nature and the concentration of the relevant allergen(s) in plasma. It should be noted that this study does not focus on the kinetics of the allergens and therefore does not address interesting questions such as the sensitivity of the setup, that is, how low concentrations of the allergen(s) that can be detected, but focus only on the dynamics of the reaction, that is, T_{react} and S_{wheal}.

If our data are transferrable to allergic patients, the results indicate that a 30-minute interval between doses in titrated oral peanut challenges in patients could be too short, particularly when the sIgE level or challenge dose is low. This is consistent with the study in peanut-allergic patients by Blumchen et al,[1] who found that 71% of their participants showed objective symptoms after more than 30 minutes, with a median T_{react} of 55 minutes using a modified oral peanut challenge protocol with a 2-hour interval between dose steps.

Limitations of this study include that the experiments were carried out in nonallergic individuals; we cannot rule out fundamental differences in the regulatory pathways of the IgE-mediated response between individuals with allergy and individuals who have been passively sensitized. Furthermore, the events taking place in the gastrointestinal tract of individuals with allergy before systemic distribution of the allergen are bypassed in this model.[7]

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Conflicts of interest: C. G. Mortz has served on a board for Novartis and has received payment for lectures from Novartis and Sandoz A/S. C. Bindslev-Jensen has received a grant from Odense University Hospital Research Foundation, has consultant arrangements with Hal Allergy and Anergis, and has received payment for lectures from Hal Allergy and Thermofisher. The rest of the authors declare that they have no relevant conflicts of interest.

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