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Early improvement in basophil sensitivity predicts symptom relief with grass pollen immunotherapy

To the Editor:

Allergic rhinoconjunctivitis and allergic asthma have a significant effect on quality of life and cause a considerable socioeconomic burden. Allergen immunotherapy (AIT) is an effective and safe treatment recommended for patients inadequately treated with symptom-relieving medication. AIT induces specific non-IgE antibodies that compete with cell-bound IgE for allergen epitopes.[1,2] Transient anergy induced in mast cells and basophil granulocytes during drug desensitization[3] and during rush subcutaneous immunotherapy (SCIT) *in vivo*[4] might offer protection until this adaptive immune response is mounted.[5] The effector cell–associated response has rarely been addressed because it has been difficult to separate from the adaptive component. Basophil sensitivity,[1,6] but not basophil reactivity,[1,7] has successfully been used to describe the development of the protective effect of SCIT as an *in vivo* allergic reaction. We hypothesized that SCIT modifies both the adaptive and cell-associated basophil response to allergen *in vitro*. To test this hypothesis, we designed an assay that separated the adaptive humoral and cell-associated components of an allergic response. We describe changes in basophil sensitivity with these assays during the first year of SCIT for grass pollen allergy.

Detailed methods can be found in the Methods section in this article's Online Repository at www.jacionline.org. Briefly, 24 adult subjects with a clinical history of grass pollen–induced rhinoconjunctivitis were randomized 3:1 to SCIT or an open control group (see Fig E1, *A*, and Table E1 in this article's Online Repository at www.jacionline.org). Basophil sensitivity ($\log_{10}$ of the allergen concentration at which half-maximal activation occurs [EC_{50}]) of the present day's cellular fraction separated from present plasma and reconstituted in present plasma, baseline plasma, or medium was measured with an allergen dilution gradient of basophil activation tests at baseline (see Fig E2 in this article's Online Repository at www.jacionline.org) every 3 weeks during a 16-week updosing and every 3 months during maintenance treatment (see Fig E1, *B*). Specific IgE, facilitated antigen binding (FAB), and IgE-blocking factor were measured at the same time points. Nasal allergen challenges and skin prick tests were done before treatment and after 1 year. Allergic symptoms and standardized medication use were recorded in diaries during the grass pollen season and were compared with a retrospective score recorded before randomization. Only local and systemic antihistamines and nasal steroids were used for symptom relief.

AIT effectively reduced symptom medication scores and increased the threshold for response in skin tests and nasal allergen challenges, 3 clinical measures of allergy (Fig 1, *A*). This was confirmed by a rapid and significant increase in grass-specific IgE levels (Fig 1, *F*). In a detailed assessment of functional protective immunity, the EC_{50} of basophils from treated patients reconstituted with present plasma increased rapidly to 5-($P = .005$), 12-, 94-, and 155-fold (all $P < .0001$) baseline sensitivity after 3, 6, 12, and 52 weeks of treatment (Fig 1, *C*, and Table I). Improvement in seasonal symptoms correlated with change in basophil sensitivity after 3 (Spearman $\rho = 0.49$, $P = .015$; Fig 1, *E*) and 6 (Spearman $\rho = 0.53$, $P = .01$) weeks when the control group and tertiles of the treatment group were compared. The early changes in basophil sensitivity at 3 and 6 weeks occur when markedly less antigen (allergen) was injected than is required to boost the immunologic memory response to vaccines. The humoral component (EC_{50} present plasma − EC_{50} baseline plasma) induced by SCIT (Fig 1, *D*) increased 4-fold ($P = .031$) after 9 weeks, 21-fold after 12 weeks, and 107-fold after 52 weeks (both $P < .0001$). The cell-associated component (EC_{50} with medium; Fig 1, *E*) increased significantly at 39 weeks ($P = .002$). Basophil reactivity (maximal percentage activation at high allergen concentration) did not change during SCIT in the current or previous studies.[6,8] In contrast, basophil sensitivity changed significantly from baseline when measured before and after the grass pollen allergen season[1,6] or once AIT was completed.[7]

FAB and IgE-blocking factor values increased significantly after 6 weeks of treatment and remained increased during maintenance treatment (Fig 1, *G* and *H*), reproducing the results

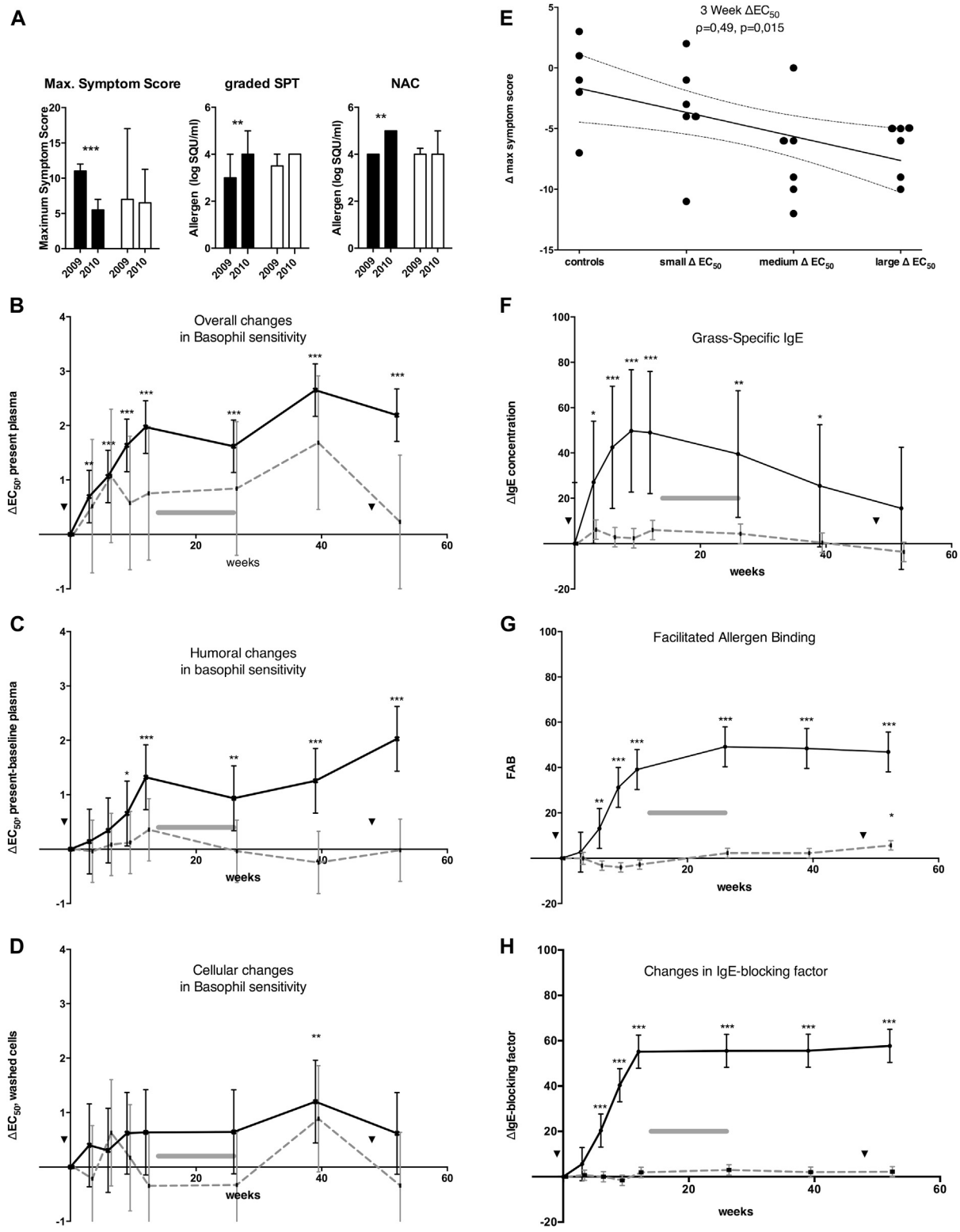

FIG 1. A, Clinical measures of treatment efficacy, maximal symptom score in season, improvement in nasal allergen challenge, and titrated skin prick tests *(SPT)*. **B-D,** Development of basophil sensitivity (means normalized to baseline with 95% CIs) of patients undergoing AIT *(solid line)* compared with control subjects *(stippled line)*. Fig 1, *B*, Basophil EC_{50} reconstituted with present plasma. Fig 1, *C*, Humoral changes (EC_{50} present plasma − EC_{50} baseline plasma). Fig 1, *D*, Cell-associated EC_{50} (washed cells in medium). Fig 1, *E*, ΔEC_{50} correlates with improvement in symptom score. Fig 1, *F*, Relative specific IgE. Fig 1, *G*, FAB. Fig 1, *H*, IgE-blocking factor. $^*P < .05$, $^{**}P < .01$, and $^{***}P < .001$. *Bar* = Symptom medication score diaries in grass pollen season. *NAC*, Nasal allergen challenge.