# Exploring the Impact of Antibody Affinity on IgE-Blocking Activity in Allergen Immunotherapy

*K. Repiská[1], G. Wozniak-Knopp[2], B. Bohle[1]*
[1]Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria, [2]Institute of Molecular Biotechnology, Department of Biotechnology, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

**Type selection**

**Choose your preferred presentation format**: Oral presentation

**General data**

**Track**: Adaptive Immunity
**Topic**: Allergen-specific immunotherapy

**Abstract text**

**Text**: Introduction: Allergen immunotherapy (AIT) induces allergen-specific antibodies (Abs) of which some bear IgE-blocking activity, mediating clinical tolerance to the administered allergen. Still, the portion of blocking Abs in all AIT-induced allergen-specific antibodies is unknown. Here, we separated allergen-specific polyclonal serum Abs by their affinity and tested whether this approach can be used to monitor the levels of IgE-blocking Abs during AIT.

Methods: mAbs of differing affinities for the major birch pollen allergen (Betv1) as determined by surface plasmon resonance were spiked into a serum from a non-allergic individual to evaluate affinity-separation with NHS-sepharose beads loaded with recombinant Betv1. Sera collected at month 6, 12, 18, and 24 of birch pollen AIT from 8 patients were incubated with Betv1-loaded beads. After centrifugation, supernatants (SN) and original sera were compared for their content of allergen-specific Abs and net-binding force by ELISA and acidic dissociation ELISA, respectively. IgE-blocking was evaluated by inhibition of allergen-induced basophil activation detected by flow cytometry.

Results: Incubation with allergen-loaded beads completely removed mAbs with a $K_D$<3 nM and partially removed mAbs with $K_D$<200 nM from serum. Accordingly, SN had a lower net-binding force than the respective sera. In contrast to sera, all SN lacked IgE-blocking activity. Consequently, the levels of non-blocking Abs in SN served to calculate the portion of IgE-blocking Abs. The latter varied individually and longitudinally but outnumbered the portion of non-blocking Abs at all time points during AIT. IgE-blocking Abs comprised more IgG4 than IgG1 and IgG3. Notably, the portion of non-blocking IgG1 increased with continuous AIT, reaching 50% in 4 out of 8 patients at month 24.

Conclusion: Our findings confirm that functional IgE-blocking depends on high-affinity Abs. Their levels change during AIT and vary individually. It is tempting to speculate that a particular threshold of high-affinity AIT-induced Abs is decisive for therapeutic success.

**Conflict of Interest statement**

**Do you have any conflict of interest to declare?**: No

**Affirmation**

**1. Consent to participate and present onsite**: Yes
**2. Consent to publication publication**: Yes
**3. Consent on treatment of contact details saved in this system**: Yes