

133 IgE AND IgG ANTIBODIES TO BEE VENOM ANTIGENS: CHANGES DURING IMMUNOTHERAPY. David M. Kemeny, B.Sc., Maurice H. Lessof, M.D., Marie McKmills, B.Sc., Guy's Hospital, London

IgE and IgG antibodies to purified proteins and peptides from bee venom were measured in sera from patients with bee sting allergy, and changes in these followed during venom immunotherapy. IgE antibodies were measured by Radioallergosorbent test (RAST) and the specificity checked by RAST inhibition. IgG antibodies were measured by a radiolabelled antigen-binding assay.

In sera from 32 bee venom allergic patients who had general reactions to bee stings, IgE antibodies were found to Phospholipase A₂ in 91%, to Hyaluronidase in 50%, and to Melittin in 39%. No IgE antibodies were found to the peptides 401 and Apamin. The highest titre of IgE antibodies in individual patients was found to be directed against Phospholipase A₂ in 69%, Hyaluronidase in 19%, and Melittin in 6% of sera. IgG antibodies were found to Phospholipase A₂ in 97% and to Hyaluronidase in 43% of sera. The titre of IgG antibody was always higher to Phospholipase A₂ even in patients whose main IgE antibody response was to Hyaluronidase.

In a patient who failed to tolerate the increased doses of bee venom given during venom immunotherapy, despite a 7 fold rise in IgG antibody to Phospholipase A₂, clinical improvement was associated with a subsequent 10 fold increase, in IgG antibody to Hyaluronidase. These results confirm that Phospholipase A₂ is the main, but not the only allergen in bee venom, and suggest that clinical improvement during venom immunotherapy may require production of IgG antibodies to all the venom antigens.

134 KINETICS OF RELEASE OF INDIVIDUAL ANTIGENS AND ENZYMES FROM SHORT RAGWEED POLLENS. R. Furic, J. Bousquet, M.D., L. Perelmutter, Ph.D., F.B. Michel, M.D., Montreal and Ottawa, Canada and Montpellier, France.

Pollens release their various proteins differently according to many conditions. The releasability of antigens E, Ra3, and Ra5, acid phosphatase (AP) and leucin amino peptidase (LAP) was assayed for up to 296 hrs. of extraction of short ragweed pollens under different extracting conditions: 50% glycerol, saline, +4°C, +22°C or after delipidation.

Antigen E was measured by the Mancini method, Ra3 and Ra5 (gift of Dr. Goodfriend) by RIA and enzymes by colorimetry.

The patterns of release of each marker was more or less similar but the amounts were different under the various conditions of extraction. Antigen E and AP were slowly released, increasing in amount from 1 to 296 hrs. Ra5 was immediately leached and was stable from 1 to 296 hrs. E/Ra5 ratio ranged from 40 to 400. Ra3 was immediately released and decreased during further extraction. LAP reached a peak at 158 hrs. and subsequently declined. The correlation between AP and antigen E was significant ($p < 0.02$) but that between antigen E and LAP was not significant.

Quantitative differences in the amount of specific allergens extracted under varying conditions of extraction may account for the variation in the quality of extracts currently available.

135 TIME-DEPENDENT RELEASE OF ALLERGENS FROM POLLEN GRAINS. PRESENCE OF MONOVALENT ANTIGENS IN THE RAPIDLY ELUTED ALLERGENS. M. Soler Ph.D., F. Anfosso Ph.D., J. Charpin M.D. INSERM U 174 Marseille (France).

Route of entry in the body of the allergens and their level of penetration are factors of importance on the expression of hypersensitivity reactions. Pollen extracts are complex mixtures of allergens which are eluted from pollen grains at different times. We studied the composition of the rapidly eluted allergens from plane-tree pollen. Plane-tree (*Platanus acerifolia*) pollen grains were suspended in PBS and aliquots of supernatants were recovered at different times. RAST was performed on sera from plane-tree sensitive patients and histamine release on their leucocytes. Desensitization of basophils was performed by incubation of leucocytes under sub-optimal conditions with the allergens, and after washing, these cells were triggered by the whole aqueous extract (WAE) or the major allergen (All.P).

Results indicated that some allergens appeared very soon in supernatants and reached their maximum rate after 15 mn of extraction. In contrast All.P was released after 2 hrs. By slicing IEF gels, 9 allergenic fractions were recovered. All of them inhibited RAST performed with WAE as solid phase. 7 out of 9 triggered basophils for histamine release. Results of basophil desensitization indicated that Fr IV ($pI=5.9$) did not cross react with All.P whereas Fr VI ($pI=6.7$) and All.P bore cross-reacting allergenic sites. Thus pollen extracts appear to be complex in terms of their content in allergens and in the valency of these allergens. Monovalent fragments will be convenient tools to study the allergenic determinants.

136 ISOLATION AND CHARACTERIZATION OF MOUNTAIN CEDAR ALLERGEN. Rachaneepas Suvunrungsi, Ph.D. and Timothy J. Sullivan, M.D., Dallas, Texas

Molecules emerging from *Juniperus sabinoides* pollen (Mountain cedar, MC) are an important cause of allergic disease in the southwestern United States. We studied the allergen(s) in aqueous extracts of MC pollen using wheal and flare skin test, RAST inhibition, and in vitro mediator release from passively sensitized human skin assays. Allergenic activity eluted from a Sephadex G-100 column in a single OD₂₈₀ peak with an apparent molecular weight of 40,000 daltons. Allergenic activity co-purified with a 40K protein during Phenyl-Sepharose (P-S), ion exchange, and lectin affinity chromatography steps. SDS-PAGE analysis of allergenic material purified by G-100 and P-S steps revealed a single detectable protein with an apparent molecular weight of 40K. Analysis of the protein and carbohydrate (CHO) content of this material indicated that the molecule was 80% to 90% CHO containing hexoses and pentoses and 10% to 20% protein. A homogenous N-terminal amino acid (AA) sequence was obtained (Asp-Asn-Pro-Ile-Asp). MC allergen (10 µg to 1 µg/ml) provoked positive skin tests and in vitro mediator release. 100% RAST inhibition was observed at 100 µg/ml MC allergen. Boiling for 10 minutes or reduction and alkylation did not destroy the skin test reactivity of MC allergen suggesting that IgE binding sites are determined by the linear AA sequence or by the CHO region. Allergic reactions to MC pollen appear to be attributable to a single, stable, readily isolated 40K glycoprotein.