737 c-DNA CLONING OF *Amb* V ALLERGENS FROM RAGWEED POLLENS. B. Ghosh, Ph.D. and D. G. Marsh, Ph.D., Baltimore, Maryland.

To study *Amb* V allergens and to determine their interactions with the human major histocompatibility gene-encoded proteins and the T-cell receptor proteins, c-DNA cloning of the allergens was undertaken. Total RNA was isolated from giant ragweed (*Ambrosia trifida*) pollen by a modification of a procedure used in plant RNA isolation. Using two degenerate oligonucleotide primers (which were synthesized based on the amino acid sequence of the N- and C-termini of *Amb t* V), a DNA fragment of 119 nucleotides was amplified using PCR technology. This has been confirmed by subcloning this 119 bp fragment into the M13 vector and subsequent sequencing by the dideoxy procedure.

In order to obtain the full-length c-DNA sequence, the above c-DNA sequence information was used to synthesize a number of gene-specific primers which were used for anchored-PCR to amplify both the 5'- and 3'-ends of *Amb t* V c-DNA. The *Amb t* V-specific amplified ends were subcloned into the M13 vector and sequenced. From the overlapping sequences of a number of independent M13 clones, the full-length c-DNA sequence was obtained and this has been used to deduce the complete amino acid sequence of *Amb t* V. This procedure can be used in general to clone allergen and other c-DNAs from complex biological sources. In fact, using two degenerate oligonucleotide primers, we have already cloned and sequenced the coding region of *Amb a* V allergen from short ragweed (*Ambrosia artemisiifolia*) pollen. Attempts are now being taken to clone the full-length *Amb a* V c-DNA using an approach similar to that described above. Both of these *Amb t* V and *Amb a* V c-DNAs will be used to generate site-directed mutants to map the Ia/T-cell epitopes and B-cell epitopes on the allergen molecules using *Amb* V-specific T-cell clones.

738 CLONING AND IMMUNOLOGICAL STUDIES OF *AMB A* II (ANTIGEN K) FROM RAGWEED POLLEN. M.C. Kuo, Ph.D., T.P. King, Ph.D., A. Brauer, B.S., R. Koury, B.S., J. Morgenstern, Ph.D., K. Keating, Ph.D., C. Counsell, B.S., X-B. Yu, M.S., I.J. Griffith, Ph.D., and B.L. Rogers, Ph.D. Cambridge, MA. and New York, N.Y.

Amb a II (formerly antigen K) is a major allergen from ragweed (*Ambrosia artemisiifolia*) pollen. *Amb a* II has been shown to be immunologically related to *Amb a* I (formerly antigen E). Recently, cDNAs encoding *Amb a* I have been cloned and sequenced and it has been demonstrated that *Amb a* I is not a single protein but products of a multigene family (Rafnar et al., J. Biol Chem., in press). Using an *Amb a* I cDNA as a probe to screen a λgt10 cDNA library as well as Polymerase Chain Reaction (PCR) methods, a full-length cDNA clone encoding *Amb a* II was obtained from ragweed flowerhead mRNA. The deduced amino acid sequence of *Amb a* II shares extensive homology (70-80%) with sequences of the *Amb a* I family of proteins.

The *Amb a* I and *Amb a* II full-length cDNAs were cloned into an *E.coli* expression vector and recombinant proteins were generated. The recombinant *Amb a* I and *Amb a* II proteins were purified from *E. coli* lysates and the specific reactivity of these proteins with IgE and T cells from ragweed allergic patients were examined. Human T cells from allergic patients were stimulated *in vitro* with purified recombinant *Amb a* I and *Amb a* II proteins in both primary and secondary proliferation assays. An extensive T cell crossreactivity was observed reflecting the high level of shared sequence of these proteins.

739 ISOLATION AND CHARACTERIZATION OF ALLERGEN *AMB A* VII FROM SHORT RAGWEED POLLEN. M. Roebber, PhD. and D.G. Marsh, PhD., Baltimore, MD.

Structure–function studies of the molecular basis of human immune responsiveness are facilitated by using: a) allergens of simple structure, toward which responsiveness tends to occur in an "all-or-none" fashion; b) protein allergens for which the three-dimensional structure is known or can readily be computed. For these reasons, we elected to isolate a highly basic, blue protein from *Ambrosia artemisiifolia* (short ragweed) pollen, which we postulated to be a plastocyanin (a copper-containing, electron-transport plant protein). This protein, and a similar colorless protein, were highly purified from an 8-min extract of defatted pollen using a combination of hollow-fiber filtration, and chromatography on Whatman DE52, Biogel P10, CM-Sephadex and hydroxylapatite columns. Both proteins gave single bands on SDS–PAGE and single immunoprecipitates against potent polyvalent antisera. The blue protein (*Amb a* VIIB) migrated slightly ahead of the colorless one (*Amb a* VIIA) on Biogel P10 chromatography (apparent M_r values = 12,000 and 11,500, respectively); but both forms exhibited the same apparent M_r values of 8,000 by SDS–PAGE. By zone electrophoresis in agarose gel at pH 8.6, *Amb a* VIIA was slightly more cathodic than *Amb a* VIIB. The B variant was slightly contaminated with the A form (suggesting partial loss of copper). The first 38 amino acids (aas) of both proteins were identified unequivocally by N-terminal aa sequencing, and were identical. Comparison against Protein Sequence Databases showed homology with plastocyanins, particularly with the 96-residue cucumber "cusacyanin" (57% identity in a 35 aa overlap). About 15–20% of ragweed-allergic individuals were found to have IgE antibodies to the *Amb a* VII proteins. Isolation of *Amb a* VII and its identification as a member of the plastocyanin superfamily for which 2 structures are known (by NMR and x-ray crystallography) will facilitate molecular and genetic studies of human responsiveness to a conserved family of plant proteins.

740 ISOLATION AND TRANSLATION OF MESSENGER RNA FROM WHITE OAK POLLEN AND INFLORESCENCES. G. Bass, M.S. and H. J. Wedner, M.D., St. Louis Missouri

Previous studies from our laboratory using single and 2D immunoblotting have demonstrated that white oak pollen contains 28 potential allergens. As an approach to the molecular characterization of these allergens, we have isolated mRNA from inflorescences (I) just prior to floral opening and from pollen (P). Total RNA was isolated by phenol/chloroform extraction and, following removal of starch by cold water extraction, mRNA was isolated by oligo-dT cellulose chromatography. The mRNA was translated in the wheat germ system and the synthesized protein separated by SDS-PAGE. mRNA from P directed the synthesis of proteins from 8 to 50KD while I mRNA gave a broader spectrum (10 to 72 KD). Synthesized proteins were blotted onto nitrocellulose and probed with a high titer rabbit anti-white oak pollen and with sera from oak sensitive patients. Both P and I mRNA produced proteins which were detected by rabbit anti-pollen protein IgE and a spectrum of proteins which bound anti-oak IgE; however, the spectrum of proteins detected by the two antisera differed. Both common and unique proteins were directed by P and I mRNA. These studies demonstrate that both P and I contain mRNA which directs the synthesis of oak pollen allergens and provides the foundation for the molecular characterization of these proteins