704 Purification of Recombinant Human Chymase Expressed in Insect Cells and Identification of Unique Cleavage Site of Self-Digestion. *Y. Kunori, T. Kawamura, K. Takagi,* Teijin Ltd., Hino, Tokyo, Japan

Human prochymase (h-prochymase) was expressed in cultured Tn5 cells infected with the recombinant baculovirus containing human-preprochymase cDNA. From the supernatant of infected Tn5 cells, h-prochymase was purified by column chromatography on gel with covalently linked soy been trypsin inhibitor (SBTI) followed by heparin sepharose. Mature chymase was prepared by a treatment with cathepsin C, which transformed h-prochymase to the active form by cleavage of dipeptide pro-segment. Purification of mature enzyme was performed by HPLC with heparin sepharose. As expected, the stoichiometric conversion of angiotensin I to angiotensin II was processed by the purified h-chymase. We also investigated the self-digestion of h-chymase reported previously (Urata *et. al,* (1990) *J. Biol. Chem.* **265,** 22348). The h-chymase was self-cleaved at a specific site under the neutral condition, although the primary sequence of the enzyme contains 16 aromatic amino acids (11 phenyl alanines and 5 tyrosines) as putative recognition sites for chymotryptic serine proteases. SDS-PAGE analysis of autolyzed h-chymase clearly showed two bands corresponding to molecular weight 12 kDa and 18 kDa, respectively. By N-terminal amino acid sequence analysis, the unique cleavage site of the self-digestion was identified to the peptide bond between Phe135 and Asn136. Further study on physiological implication of h-chymase self-digestion is in progress.

705 Transatlantic Transfer of Digitized Antigen Signal by Telephone Link. *J. Benveniste, P. Jurgens, W. Hsueh and J. Aissa.* Digital Biology Laboratory (DBL), 32 rue des Carnets, 92140 Clamart, France and Northwestern University Medical School, Chicago, IL 60614, USA.

Ligands so dilute that no molecule remained still retained biological activity which could be abolished by magnetic fields [1-3], suggesting the electromagnetic (EM) nature of the molecular signal. This was confirmed by the electronic transfer to water (W) of molecular activity, directly or after computer storage [4-7]. Here, we report its telephonic transfer. Ovalbumin (Ova), or W as control, were recorded (1 sec, 16 bits, 22 kHz) in Chicago using a transducer and computer with soundcard. Coded files were transferred to DBL's computer as e-mail "attached documents." Digitally amplified, they were replayed for 20 min to W (dOva, dW), which was then perfused to isolated hearts from Ova-immunized guinea-pigs. DBL staff were blind though technical incidents revealed the codes of 4/19 files to the computer operator. Coronary flow variations were (%, mean ± SEM, nb of measures): naive W (negative control), 4.9 ± 0.3, 41; dW, 4.4 ± 0.3, 58; dOva, 24.0 ± 1.4, 30, p = 4.5 e-17 vs dW; Ova (0.1 μM, positive control), 28.9 ± 3.7,19, nsvs dOva. The hitherto neglected physical nature of the molecular signal emerges: EM radiation under 22 kHz that can be digitized, transferred long distances and replayed to W, which then acquires the source-molecule's activity. This implies novel strategies in chemistry, biology and medicine. [1] Davenas et al., Nature. 1988, 333:816; [2] Benveniste et al., C R Acad Sci Paris. 1991, 312:461; [3] Benveniste et al., FASEB J. 1992, 6:A1610; [4, 5] Aissa et al., FASEB J. 1993, 150:A146 & 1995, 9:A425; [6] Thomas et al., FASEB J. 1996, [7] Benveniste et al., FASEB J. 1996, 10:A1479.

706 Identification of HLA-DQ6 and HLA-DQ8 Restricted T-cell Determinants on House Dust Mite, Ryegrass and Ragweed Allergens. *S Chapoval, CJ Krco, L DeRosa, J Harders and CS David.* Department of Immunology, Mayo Clinic, Rochester, MN.

We have investigated the genetic and molecular basis of specific immune responsiveness to house dust mite *(Dermatophagoides pteronyssinus; Der p),* ryegrass *(Lolium perenne; Lol p)* and short ragweed *(Ambrosia artemisiifolia; Amb a)* allergens using transgenic mice expressing DQ8 (HLA-DQA1*0301, HLA-DQB1*0302) or DQ6 (HLA-DQA1*0103, HLA-DQB1*0601) genes. Panels of overlapping 20mer peptides which mapped primary amino acid sequences of the major antigens *Der p2, Amb a2, Amb a5* and *Lol p3* were synthesized with whole extract or individual peptide subcutaneously and lymph node cells were challenged *in vitro.* Strong HLA-DQ8 restricted responses were detected to several peptides of *Der p2* (1-20, 41-60, 51-70, 61-80, 91-110, and 101-120) and *Lol p3* (1-20, 31-50, 51-70, 61-80, 71-90, and 81-97). In contrast T cells of HLA-DQ6 mice recognized fewer peptides of these allergens. High levels of T-cell proliferation were found in HLA-DQ8 mice in response to peptides 1-20, 11-31, and 21-40 of *Amb a5,* while HLA-DQ6 mice exhibited undetectable responses to peptides 21-40 and 31-45, and reacted moderately to peptides 1-20 and 11-31. HLA-DQ6 mice showed strong responses to some epitopes of *Amb a2* molecule.

These results demonstrate the specificity of HLA class II polymorphism in allergen sensitivity and pave the way for developing antagonistic allergen peptides for desensitization.

707 Oral Tolerance in Protein- and Hapten-Induced Active Fatal Anaphylaxis. *HK Lee, JS Park, and TY Ha.* Dep. of Immunology, Chonbuk Natl. Univ. Med. Sch. Chonju, 561-182. Rep. of Korea

This study was undertaken to investigate whether oral intake of antigens could prevent the active systemic anaphylaxis induced by ovalbumin (OVA) or penicillin V (PEV) in C57BL/6 mice. OVA induced anaphylaxis was completely prevented by single feeding of OVA before sensitization. This procedure also significantly inhibited OVA-specific IgE and IgG responses. The reaction was not prevented when the animals were fed simultaneously with or after sensitization. Inability of spleen cells from tolerant donors to transfer the tolerance to naive recipients, and no inhibition of proliferation of spleen cells from OVA-sensitized donors by the addition of tolerant cells, argued against the role of suppressor cells. Tolerant spleen cells were neither proliferate nor produce IL-2 in response to OVA, but the tolerant state was reserved by culturing the cells in the presence of IL-2, demonstrating anergy as one of the mechanisms underlying oral tolerance in this system. PEV-induced fatal anaphylaxis could be prevented by feeding of the carrier protein conjugate. Oral tolerance in protein- and hapten-induced anaphylaxis could offer the clinical basis for attemting a new strategy in the prevention of anaphylaxis.