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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 Phel35 and Asnl36. 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-ping 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.

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 Phel35 and Asnl36. Further study on physiological implication of h-chymase self-digestion is in progress.

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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.

Poster

TRANSATLANTIC TRANSFER OF DIGITIZED ANTIGEN SIGNAL BY TELEPHONE LINK

*J. Benveniste, J. Aïssa, P. Jurgens and W. Hsueh**

INTRODUCTION

Agonists so dilute that no original molecule remains still express their specific activity [1-4]. The latter was abolished by magnetic field [3], suggesting an electromagnetic nature for the molecular signal. Its transfer to water via electronic circuitry or after computer storage was then shown [5-12].

We now report the transfer of a digitized antigen signal by telephone link.

MATERIALS AND METHODS

Immunization. Male Hartley guinea-pigs, 300 g, were injected s.c. with 1 µg ovalbumin (Ova) from Sigma, in 0.1 ml alum (Alhydrogel®, Superfos Biosector a/s).

Heart preparation. Isolated hearts were perfused according to the classical Langendorff method using Krebs-Henseleit buffer (pH 7.4) gassed with O2/CO2, 95/5 %, at a pressure of 40 cm H2O at 37 C. Samples were injected (2 ml) via a catheter just above the aorta at about 1/10th of the coronary flow (circa 5 ml/min).

Recording and transfer. Plastic tubes (15 ml, Falcon) containing ovalbumin (Ova), acetylcholine (ACh), dextran, or water were used as source. Recording (1/2 or 1 sec, 16 bits, 22 kHz, one floppydisk per file) was performed in Chicago using a purpose- designed transducer and a computer equipped with a sound-card. Coded files, recorded on disks, were sent to the Digital Biology Laboratory (DBL) in Clamart:

1. In a preliminary experiment (Table 1), disks were sent by regular mail.

2. In subsequent experiments (Tables 2 & 3), they were transferred as e-mail "document attached" to the DBL's computer, and directly downloaded onto disks (one disk per file). Files were digitally amplified and "played" using the computer sound-card, onto 15 ml water tubes for 20 min (2.5-5 volts AC).

Assays. Water, appropriately pre-exposed to the specific signal of digital Ova, ACh or water (d-Ova, d-ACh, d-water), was then infused to isolated hearts. Untreated (naive) water and Ova (0.1 µM) were infused as negative and positive controls respectively. Coronary flow was then measured every min for 30 min and changes were calculated as % maximal coronary flow variation compared to three "time 0" values.

Results were e-mailed to Chicago and code keys were sent in return.

Mechanical parameters (min. and max. tension, heart rate) were observed and recorded using dedicated software (*Emka Technologies*).

Student's t test for unpaired variates (Plot 40, Sigma Plot) was used to assess statistical significance.

RESULTS

Files were always blind to the heart operator (see legend of Table 3). They were submitted to analysis whatever the experimental conditions and even when hearts reacted poorly to Ova 0.1 µM.

The effects on the maximal variation in coronary flow of perfused hearts from Ova-immunized guinea-pigs are shown in Tables 1, 2 & 3.

All results are given as % variation in coronary flow, % CFV (+ SEM when applicable).

TABLE 1

Expt #1: Four coded, digitally recorded files (May 1996) Q, S, X, and W were sent via air-mail from Chicago to Clamart.

key	% CFV	Answer from DBL	Activity recorded
Q	5.6 8.3	Water ?	Water
S	17.1	Ova ?	Ova
X	5.7 27.8	Ova ?	Ova
W	3.1 6.2	Water ?	Water

Naive water: 2.9: Ova 0.1 µM: 30.3

TABLE 2

Expt #2: Six coded, digitally recorded files (June 1996, were sent via e-mail from Chicago to Clamart.

key	% CFV (mean +- SEM) n = 4-6	Answer from DBL	Activity recorded
21	4.9 +- 0.5	No effect : Water?	Water
22	20.9 +- 2.8	Effect : Ach?	Ach
23	22.4 +- 1.8	Effect : Ach?	Ach
24	6.7 +- 0.7	No effect : Water?	Water
25	5.9 +- 0.9	No effect : Water	Water
26	21.4 +- 7.2	Effect : Ach?	Ach

Naive water: 4.7 + 0.3, n = 16; ACh 0.1 µM: 21.1 + 1.5, n = 12

TABLE 3

Expt #3 (August-September 1996)

Expt No.	Key	% CFV measured blind * in DBL	n=	Activity recorded in Chicago
1	C2	20.0 23.1 44.4	3	Ova
	C5	1.6 3.1	2	Water
2	C4	24.6 25.8	2	Ova
	C6	2.1 2.1	2	Water
3	C7	22.1 +- 6.1 (mean +- SEM)	4	Ova
	C9	3.3 +- 0.7	5	Water
4	C8	24.4 11.9 13.0	3	Ova
	C10	2.4 4.8	2	Water
5	C1	24.0 +- 2.2	5	Ova

	C3	4.1 +- 0.9	8	Water
6	C11	35.7 34.8	2	Ova
	C16	4.9 +- 0.8	4	Water
	C25	6.0 +- 0.4	4	Empty tube
7	C21	23.9 +- 2.4	7	Ova
	C22	7.7 +- 2.6	5	Water
	C23	4.6 +- 1.1	6	Dextran
8	C18	4.4 +- 0.4	8	Dextran
	C19	24.7 18.2	2	Ova
	C20	4.1 +- 0.6	6	Water

All results:

d-Ova 24.0 + 1.4 (n = 30) p = 4.5 e-17
d-water 4.4 + 0.3 (n = 58)

Controls:

Naive water 4.9 + 0.3 (n = 41); ns compared to d-water.

Ova 0.1 µM 28.9 + 3.7 (n = 19); ns compared to d-Ova.

* Files were always blind to the heart operator. However, due to technical incidents, the content of C4/C6 and C8/C10 were known to the computer operator when played to water.

Comments. d-Ova, d-ACh and Ova (0.1 µM), induced highly significant changes on coronary flow, whereas naive water- and d-water-induced effects were indistinguishable from spontaneous flow variations. By contrast, d-Ova, as well as Ova 0.1 µM, had no effect on hearts from non-immunized animals (results not shown). The difference in coronary flow variation following injection of d-Ova vs the controls, water and d-water, was highly significant.

d-Ova influenced heart mechanical parameters only when hearts reacted well to Ova 0.1 µM. Representative mechanical effects are shown in Figs 1 & 2.

Fig.1

Effect of d-Ova on maximal tension (g)
(September 20, 1996)

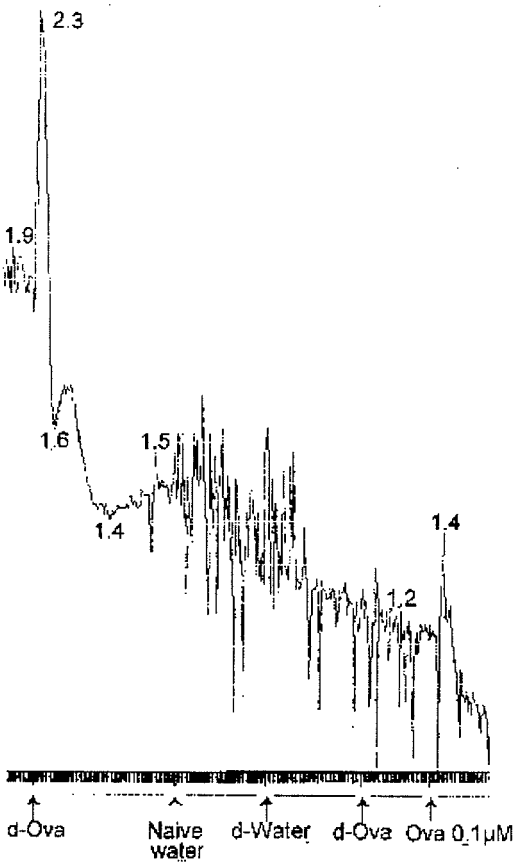
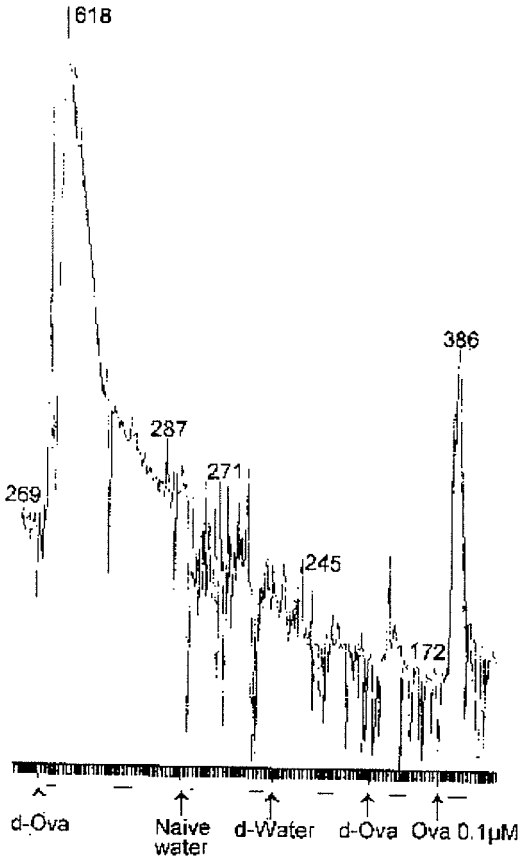


Fig.2

Effect of d-Ova on heart rate (bpm)
(September 20, 1996)



DISCUSSION

Using a purpose-designed transducer and a sound-card-equipped computer, we digitized, recorded and "replayed" to naive water two signals embodying, respectively, the activity of a small molecule, ACh (MW 182) and of a larger one, Ova (43 Kd).

Treated water influenced cardiac function as did the original molecules.

The specificity of digitized activity is evident in the fact that recorded water (d-water) and dextran were ineffective on hearts from Ova-immunized animals whereas d-Ova, fully active on hearts from immunized animals, had no effect on those from non-immunized animals.

These results demonstrate that at least some biologically active molecules are capable of transmitting their activity to water that can be recorded, digitized and replayed in the form of electromagnetic radiation of less than 22 kHz.

We therefore suggest that:

1.The molecular signal consists of low frequency waveforms.

2.One of the physiological roles of water is to mediate this signalling (an hypothesis supported by contemporary physical theory [13]).

3.The digitized signal can be "replayed" to water where it subsists independently.

4.As could be expected, digitized files can be transferred via telephone link.

This opens the way to:

.Transmission of biological (and possibly chemical) specific signalling,

.The immediate application of digitized molecular activities in biology and medicine,

.Upon elucidating the signal, its potentiation and modification to produce de novo therapeutic activities not associated with any known molecule,

.Protocols for digital detection and diagnosis.

CONCLUSION

Transfer via telephone link of the molecular signal, composed of waveforms in the 0-22 Khz range, opens the way to purely digital procedures for the analysis, modification and transmission of molecular activity, with clinical and possibly industrial applications.

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