MATERIALS AND METHOD: Acarosan®-deep cleaner solution with 5.7% solidified BB was applied in the laboratory for the control of up to 60.000 mites living in 5 carpet pieces of 2500 cm^2 in area. After application, a concentration of 3g $BB/m^2$was present in the carpets. No removal of the liquid cleaner was performed. The carpets were followed for a maximum period of 12 weeks.

RESULTS: In all carpets, living mites numbers were reduced after 1 day to 98.8 to 99.4%. After 8 days, a reduction of 99.4 to 99.9% was observed. 12 weeks after application of the deep cleaner formula, a reduction of 99.9% was still present.

DISCUSSION: Mites can be killed for up to 12 weeks with an acaricidal deep cleaner solution. The concentration of BB in the carpet after deep cleaning is reduced to 0.9 g/m^2 from an original 3 g/m^2. From previous experiments, we know that a concentration of at least 0.5 g BB/m^2 carpet is sufficient to kill all mites present. Many studies are on going which show the effect of deep cleaning on total soil removal including allergens. Further experiments will show the effect of deep cleaning on the reduction of allergenic dust in particular.

962 Potentiation of Immunogenicity and Modulation of Lymphokine Profile in House Dust Mite (Derf2) Specific T Cells by Genetically Modified Allergen *S Korematsu*†, Y Tanaka†, Y Okumura‡, S Hosoi*, N Minato†* *Department of Pediatrics and Developmental Medicine, Graduate School of Medicine, Kyoto University †Laboratory of Immunology and Cell Biology, Graduate School of Biostudies, Kyoto University ‡Research and Development Laboratory, Asahi Breweries Ltd

The most common causative agents for atopic allergy in Japan are house dust mites. In this study, we established several T cell clones specific for one of the group 2 allergens, Derf2, derived from *Dermatophagoides farinae*, and examined the possibility for the specific hyposensitization therapy by using genetically engineered Derf2 (C8/119S), whose cystein residues at positions 8 and 119 were substituted for serine residues respectively, and reactivity to human IgE was largely abolished in in vitro binding assay and skin prick tests.

Derf2 specific CD4 positive T cell clones were established from antigen boosted peripheral blood mononuclear cells (PBMC) of two mite allergic patients by the standard limiting dilution method. C8/119S induced proliferation in the T cell clones by far more efficiently than Derf2 in terms of the required antigen concentration for the half maximal responses (10-1,000 times) as well as the maximal responses (more than two times) in 3 out of 4 independent clones. The greatly potentiated immunogenicity of C8/119S was suggested to be due to the altered supramolecular structures. Furthermore, C8/119S preferentially stimulated Th1 type T cells in PBMC of allergic individuals while Th2 cells were induced in case of Derf2. The difference could be ascribed to the inability of B cells in allergic individuals to present antigens selectively for C8/119S.

These results collectively suggest that C8/119S mutation of Derf2 leads to the potentiated immunogenicity and Th1-shifted lymphokine induction by altered supramolecular structure and loss of IgE reactivity, respectively. We thus conclude that C8/119S is a suitable immunogenic protein for the antigen specific hyposensitization therapy.

964 Skin Test Reactivity to Natural and Recombinant Mite Allergens *F Caballero*†, M Sánchez-Borges*, M Bernstein*, O Aldrey*, M Chapman†, E Fernández-Caldas‡* *Caracas, Venezuela †University of Virginia, Charlottesville, Virginia ‡CBF Leti, Madrid, Spain

In order to compare the allergenic activity of natural and recombinant mite allergens, 92 allergic patients (66 female, 26 male, mean age 29.6 ±13 years) and 30 non allergic subjects were prick-tested with 1:10, 1:100, 1:1000 and 1:10000 dilutions of *D pteronyssinus* (1000 AU/ml) and B tropicalis (2000 mcg/ml) extracts (from CBF Leti,Madrid), as well as with recombinant Der p5 (5 mcg/ml) and Blo t5 (5 mcg/ml) allergens.Skin test results to 1:10 dilution are summarized as follows:

	DER P	DER P5	BLO T	BLO T5
%POSITIVE$	83.7	38.0*	73.9	42.4*
WHEAL SIZE	5.64	2.43	4.22	1.82*

*P <0.05, $ WHEAL 3 MM

None of the control subjects reacted to any of studied antigens.These results suggest that in spite of the high prevalence of mite sensitivity present in this allergic population, skin test to Group 5 allergens is significantly lower when compared to natural mite allergen extract.

965 Structural Analysis of Jun a 3, a Potentially Inducible Allergen of Mountain Cedar Pollen *TM Midoro-Horiuti, RM Goldblum, CH Schein, KV Soman, W Braun, A Kurosky, EG Brooks*

The increasing prevalence of allergic diseases in industrialized countries may be due to changes in environmental factors which may modify allergens or the responses to them. The expression of plant proteins known as pathogenesis-related (PR) proteins, are upregulated under certain environmental conditions, including pollutants exposure.

Hypersensitivity to mountain cedar (*Juniperus ashei*) pollen is a frequent cause of severe seasonal allergic disease. We have cloned and sequenced the major allergens of the mountain cedar pollen, Jun a 1 and Jun a 3, using a PCR based method and a cDNA library generated from mountain cedar pollen. Jun a 1 consists of 346 amino acids and has a high degree of homologous with Japanese cedar (Cryptomeria japonica) allergen Cry j 1, Japanese cypress (Chamaecyparis obtusa) allergen Cha o 1 and several pectate lyases.

The second mountain cedar allergen Jun a 3 is homologous to the thaumatin-like PR plant protein group 5, but its sequence is not related to any known pollen allergens. PR-5 proteins are typically induced in plants under stress including infections, drought and expose to environmental pollutants. The amount of Jun a 3 varied up to five-fold in pollen from the same region collected in different years, and from those of different regions during the same year. Thus, the allergenicity of plant pollens that express PR-proteins may vary greatly with the environment of the plants.

A 3-D model structure of Jun a 3 was built on the crystal structures of homologous proteins, thaumatin and an anti-fungal protein from tobacco. The IgE epitopes of Jun a 3 were identified by dot blot analysis of the HPLC fractions of tryptic digests. Each fractions was dotted on the membrane and incubated with pooled serum from patients with Jun a 3 hypersensitivity. The peptide fragments in the dot blot-positive fractions were identified by mass-spectrometer.

Three IgE epitopes were located on an α-helix/turn region on the surface of one face of the model structure. No epitopes were identified from the remaining β-sheet region of the molecule.

A more complete characterization of the structure of these allergens may help to explain their allergenicity and provide new approaches for immunotherapy for this potential environmentally inducible pollen allergen.