

Allergenigenicity of Hybrid Ag 5s of Yellow Jacket and Paper Wasp Venoms

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Hybrid Ag 5 · Venom, yellow jacket · Venom, paper wasp

Introduction

Antigen (Ag) 5 is a major allergen of vespoid venoms. Homologous Ag 5s from yellow jacket (*Vespula vulgaris*) and paper wasp (*Polistes annularis*) have 59% sequence identity of their respective 204- and 205-amino acid residues. These two Ag 5s, designated as Ves v 5 and Pol a 5, respectively, have low degrees of antigenic cross-reactivity in insect-allergic patients and in animal models [1]. The structure of Ves v 5 was solved recently by X-ray crystallography [2]. We have prepared hybrids containing different segments of these two vespoid Ag 5s by expression in yeast and studied their immunological properties.

Preparation and Characterization of Hybrid Ag 5s

We reported previously that recombinant Ves v 5 and Pol a 5 from expression in yeast (*Pichia pastoris*) retained the native structure of the natural protein [3]. This is in contrast to the expression in bacteria that the recombi-

nant Ag 5s were denatured proteins [4]. The yeast expression system was used for preparing hybrids of these two Ag 5s. Hybrids PV1 and 3 contain, respectively, the N- and the C-terminal ¼ of the Ves v 5 molecule together with portions of Pol a 5 to complete the hybrid. hybrid VP3 has the opposite arrangements of the two Ag 5 fragments, when compared to PV3. The size and purity of hybrids were established by gel electrophoresis and mass spectrometry. Circular dichroism data suggested that the hybrids had the native structure of natural Ag 5.

Antigenicity and Immunogenicity of Hybrids in Mice

Antigenicity of hybrids was tested by inhibition ELISA of Ves v 5-specific antibodies on solid-phase Ves v 5. At the highest concentration of 50 µg/ml tested, Ves v 5, PV1, PV3 and VP3 showed maximal inhibitions of 100, 67, 14 and 87%, respectively. The observed inhibition by the hybrids was not due to cross-reacting epitopes of the Pol a 5 portion of the molecule as the sample of Ves v 5-specific antibodies used for inhibition studies was depleted of Pol 5-cross-reactive antibodies.

The concentrations of hybrids required for half-maximal inhibition were about 100 times higher than of Ves v 5. This difference does not reflect that the hybrids lack the native structure of Ves v 5 as the denatured Ves v 5

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from expression in bacteria did not show such inhibition. The difference in the inhibitory activities of Ves v 5 and hybrids is related probably to their epitope densities. Epitope density can influence strongly the affinity constant of a multivalent antigen and a bivalent antibody [5].

The hybrids differed in their immunogenicity to induce Ves v 5- or Pol a 5-specific antibodies in mice. PV1 was more immunogenic for the Ves v 5 response than PV3 was. But PV3 was more immunogenic for the Pol a 5 response than PV1 was. These findings support the above inhibition data that the N-terminal region of vespid antigen 5 was an immunodominant epitope.

Proliferation assays were made with spleen cells from mice immunized with hybrids. As expected, the hybrids induced T cell responses for both Ag 5s.

Allergenicity of Hybrids in Patients

This was tested by a histamine release assay with basophils from ten yellow jacket-sensitive patients. PV1 was tested only in 4 of these patients and PV3 and VP3 were tested in all patients. The results indicate that the hybrids PV1 and PV3 were, respectively, 10^2 - to 10^3 - and 10^3 - to 10^4 -fold less allergenic than Ves v 5 was, but VP3 did not show a decrease. Only 1 patient tested showed greater allergenicity for the hybrids PV1 and 3 than for Ves v 5, but this patient was more sensitive to Pol a 5 than to Ves v 5.

The allergenicity of recombinant Ves v 5 from bacteria was compared with those of the natural Ves v and the recombinant ves v 5 from yeast. In 3 patients tested, the recombinant protein from bacteria was about 10^3 times less potent than the natural protein or the recombinant protein from yeast.

Allergenicity depends on the interaction of a multivalent allergen with cell-bound IgE antibodies. The decrease

in allergenicity of the hybrids was due to a reduction of the discontinuous epitopes, as they had an ordered structure similar to that of Ves v 5. However, the decrease in allergenicity of the recombinant Ves v 5 from bacteria was due to loss of the discontinuous B cell epitopes as it had a disordered structure.

Conclusion

Our immunological findings of the hybrids in mice showed the presence of the discontinuous B cell epitopes in the N- and C-terminal regions of Ag 5 and the immunodominance of the epitope(s) in the N-terminal region. These findings suggest that the hybrids are useful reagents to map the discontinuous B cell epitope-containing regions of proteins. At present the only known way to map discontinuous B cell epitopes is by X-ray crystallography of Ag-Ab complexes [6].

Our results demonstrate that hybrid allergens can have a 100- to a 1,000-fold reduction in allergenicity, yet retaining the immunogenicity of the natural allergens. This reduction in allergenicity of hybrids is due to a decrease of B cell epitope density. Each of the hybrids we have studied has only a portion of the B and T cell epitopes of Ves v 5, and a mixture of the hybrids can, in principle, reconstitute the complete epitope library. Thus, this may be a useful approach to prepare modified allergens for use as vaccines as many allergens have sequence homology with proteins from other sources.

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