

A Naturally-Processed Universal T Cell Epitope Is Located Within a Conserved Region of Der p 2: Implications for Allergen Sensitization and Immunotherapy

Bo Wu Luc Vander Elst Marc Jacquemin Jean-Marie R. Saint-Remy

Center for Molecular and Vascular Biology, University of Leuven, Belgium

Key Words

Der p 2 · T cell epitope · Allergen sensitization

Abstract

Background: Major allergens could represent a 'port of entry' to allergen polysensitization. The immunogenic properties of Der p 2 T cell epitopes were evaluated to test this hypothesis. **Material and Methods:** T cells were obtained from regional lymph nodes of mice belonging to four different haplotypes and used in proliferation assays using Der p 2 or synthetic peptides. **Results:** T cells from recombinant (r) Der p 2-immunized Balb/c mice (H-2^d) proliferated to an immunodominant region encompassing residues 21–35, which also stimulates T cells of mice expressing three alternative haplotypes (H-2^b, H-2^k and H-2^s), indicating the presence of a universal T cell epitope. Epitope mapping identified Ile28 as a critical H-2-binding residue for all four different haplotypes. p21–35 presents a sequence homology with FIS, another universal T cell epitope. FIS priming boosts immune responses to distinct weak immunogens. Balb/c mice primed with p21–35 or FIS produced stronger immune responses to transferrin upon administration of the latter than unprimed mice. Besides, this procedure profoundly affected the overall B cell response to rDer p 2. **Conclusion:** An immunodominant T cell epitope of Der p 2

behaves as a universal epitope and enhances responses to related and unrelated coadministered antigens. These findings have implications for the understanding of allergen polysensitization and for immunotherapy.

Copyright © 2001 S. Karger AG, Basel

Introduction

Allergens are defined as major or minor according to the frequency of recognition by sensitized individuals. Genetic and/or environmental factors probably play a role in sensitization to such major allergens. The situation is, however, much more complicated when evaluating the whole anti-allergen immune response, including minor allergens.

We hypothesized that major allergens are in fact the first allergens to be recognized, representing a sort of 'port of entry' in allergic sensitization. This somehow presupposed that major allergens exhibit intrinsic properties through which sensitization could occur to minor, weaker or less frequently encountered allergens [1]. This second stage in allergen sensitization could result from simple environmental factors such as allergen exposure and be independent of the host genetic background. If proven to be true, such a mechanism would help understanding how polysensitization develops and possibly how sensitization

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2001 S. Karger AG, Basel
1018–2438/01/1243–0383\$17.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. Jean-Marie R. Saint-Remy
Center for Molecular and Vascular Biology
University of Leuven, Campus Gasthuisberg, O&N, Herestraat 49
B–3000 Leuven (Belgium), Tel. +32 16 345 791
Fax +32 16 345 990, E-Mail jeanmarie.saint-remy@med.kuleuven.ac.be

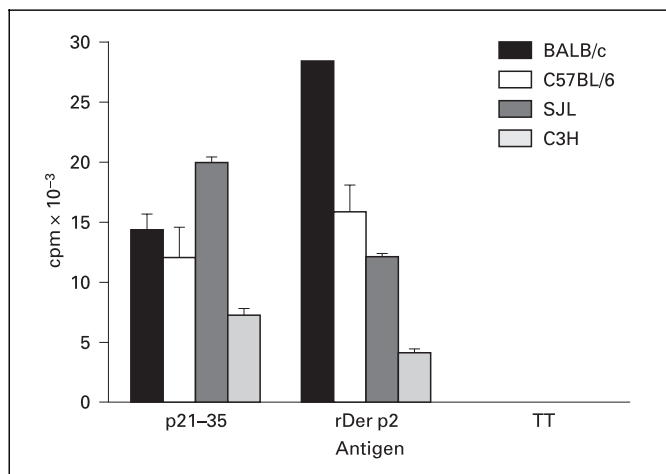


Fig. 1. Groups of 6 mice expressing different haplotypes were immunized with p21–35. Pooled lymph node cells were stimulated in vitro with either p21–35, rDer p 2 or tetanus toxoid (TT) as a control. Results are expressed as mean $\pm$ SD of triplicate determinations after subtracting background values.

could be prevented or eliminated through specific immunotherapy [2].

We have recently explored the immune properties of a major *Dermatophagoides pteronyssinus*-derived allergens, Der p 2, and found that one of the major T cell epitopes fulfills the criteria for such a role.

Material and Methods

The cDNA of Der p 2 was prepared by reverse transcriptase from mRNA obtained from cultures of *D. pteronyssinus* and cloned into pPICZαC for expression in *Pichia pastoris*. Recombinant (r) Der p 2 was found to react with a panel of conformation-dependent mouse monoclonal antibodies. Cross-inhibition studies showed rDer p 2 to be similar to native Der p 2.

Mice were immunized by footpad injections of rDer p 2 in complete/incomplete Freund's adjuvants. T cells were prepared from regional lymph nodes. They were used in proliferation assays in which rDer p 2, or a peptide thereof, was presented by autologous spleen-derived adherent cells. Synthetic peptides were purchased from Eurogentec (Seraing, Belgium).

Results

rDer p 2 elicited vigorous immune responses in Balb/c (H-2^d) mice. Proliferation assays carried out with sets of 15-mer peptides covering the entire Der p 2 sequence identified two main T cell epitopes located in positions

Table 1. Effect of priming and coadministration of p21–35 and/or FIS on rDer p 2 and transferrin immunogenicity

Priming	rDer p 2	Transferrin
p21–35	1/320	1/320
FIS	1/640	1/640
PBS	1/80	1/40

Three groups of mice were primed with PBS, p21–35 or FIS, respectively. Immunization was carried out using rDer p 2 together with PBS, p21–35 or FIS. Another three groups of mice were primed with PBS, p21–35 or FIS, respectively, and immunization was carried out using transferrin together with PBS, p21–35 or FIS. Specific IgG to rDer p 2 and transferrin are shown as serum dilution. Each value represents a mean of pooled sera from 5 mice.

21–35 and 71–85, respectively. Three alternative H-2 haplotypes were used in the same assays, including the C57BL/6 (H-2^b), a strain classically considered as high responder, C3H (H-2^k) and SJL (H-2^s). A strong proliferation was obtained in each case with p21–35 (CHGSEP-CIIHRGKPF) (fig. 1).

As p21–35 appeared to be universal insofar as it can be presented in the context of several distinct H-2 haplotypes, MHC-anchoring residues were localized by using predictive algorithms. Ile in position 28 could correspond to position 4 and represent a residue for a supermotif. Peptides with Ile28 substituted by Ala or Thr were produced and showed complete abolition of the proliferation of T cells obtained from mice of each of the four haplotypes tested.

Sequence homology searching for p21–35 identified another peptide from sperm whale myoglobin, FIS, which also has universal properties and shares the sequence SE-X(X)-IIH with p21–35. However, in addition to such property, FIS has been demonstrated to boost immune responses towards distinct antigens administered concomitantly, including weak immunogens. We, therefore, examined whether priming with p21–35 would produce similar effects. Thus, Balb/c mice were primed with p21–35 and then immunized with either rDer p 2 or an unrelated antigen, transferrin. It was observed that such priming resulted in the recognition of B cell epitopes scattered over the entire Der p 2 molecule with a 4-fold increase in the titer of specific antibodies. Besides, the immune response towards transferrin was increased by 8-fold, a result comparable to that obtained when priming was carried out with FIS (table 1).

Discussion

A region of Der p 2 encompassing residues 21–35 presents the characteristics of a universal T cell epitope, being recognized in the context of at least four different mouse H-2 haplotypes. Interestingly, this recognition is mediated through the use of a H-2-anchoring residue represented here by Ile28 and constituting a supermotif. p21–35 is located within a conserved region of Der p 2 [3],

which indicates that the results reported here are also applicable to other group 2 allergens, such as those of *Dermatophagoides farinae* and *Dermatophagoides microceras*. The fact that priming with p21–35 strongly affects the overall immune response towards Der p 2 and, in addition, can boost the production of antibodies of distinct specificities makes it possible that Der p 2 sensitization facilitates immune responses towards other allergens to which individuals are exposed at the same time [4].

References

- 1 Silvestri M, Rossi GA, Cozzani S, Pulvirenti G, Fasce L: Age-dependent tendency to become sensitized to other classes of aeroallergens in atopic asthmatic children. *Ann Allergy Asthma Immunol* 1999;83:335–340.
- 2 Des Roches A, Paradis L, Menardo JL, Bouges S, Daures JP, Bousquet J: Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. VI. Specific immunotherapy prevents the onset of new sensitizations in children. *J Allergy Clin Immunol* 1997;99:450–453.
- 3 Stewart GA: Dust mite allergens. *Clin Rev Allergy Immunol* 1995;13:135–150.
- 4 Koch YY, Choi JW, Lee MH, Sun YH, Lee SI, Lee SY, Oh JW: A preceding airway reaction to one allergen may lead to priming of the airway responses to another allergen. *Allergy* 1997;52:284–292.

Copyright: S. Karger AG, Basel 2001. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.