

CD8 T Cell Responses to Allergens and Peptides in Humans

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Key Words

CD8 T cells · CD8 T cell epitopes · House dust mite · Der p 1 · Anergy · Interferon-gamma · Specific immunotherapy

Introduction

CD8 T cells are important immunoregulatory cells in animal models of allergic disease [1], but their role in human allergic responses has not been well defined. Early human studies did suggest the generation of CD8 'suppressor' T cells following allergen-specific immunotherapy (SIT), although more detailed recent research has not been forthcoming. Although CD8 T cells have now been shown to produce a number of cytokines, interferon-gamma (IFN- γ) remains an important product [2]. Recent publications have shown that, in some situations, IL-4 and IL-5 can also be produced [2] but, in general, the immunoregulatory activities of CD8 T cells are mediated by IFN- γ [1, 3]. Human CD4 T cell epitopes on Der p 1 have been studied by a number of authors and, although epitopes were present throughout the molecule, the region between amino acid positions 111 and 139 contained the major immunodominant epitope(s) [4–6]. There have been no studies published on the characterization of

human CD8 T cell epitopes in Der p 1, although in mice, the Der p 1 sequence 111–119 was identified as a possible Db- or Kb-restricted epitope based on putative MHC class I-binding motifs [7]. Furthermore, immunization of C57BL/6 mice with Der p 1 peptide (p) 111–139 induced specific CD8 responses with high IFN- γ production. In this report, we studied the in vitro allergen-specific responses of human CD8 T cells to Der p 1 in *Dermatophagoides pteronyssinus* (Dpt)-allergic and nonallergic subjects. Using overlapping synthetic peptides, we also investigated CD8 T cell epitopes on the Der p 1 molecule.

Patients and Methods

Patients

All atopic patients were symptomatic and sensitive to Dpt by skin prick test. Ten of the house dust mite (HDM)-sensitive patients had received Dpt SIT performed by subcutaneous injection using Allpyral Dpt extracts (Bayer). For the desensitization course, patients received 9 fortnightly injections reaching a final dose of 1.0 ml of the 10,000-PNU/ml extract, followed by monthly maintenance injections of 0.5 ml of the 10,000-PNU/ml extract. All post-SIT patients had received therapy for at least 12 months.

CD8 T Cell Isolation and Culture

PBMC were isolated on Ficoll-Hypaque and positive isolation of CD8 T cells was undertaken using Dynal immunomagnetic beads coupled with anti-CD8-specific monoclonal antibody according to

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Table 1. Mitogen- and allergen-specific release of IFN- γ from PBMC and CD8 T cell cultures

Clinical status	PBMC			CD8 T cells		
	PHA	Der p 1		PHA	Der p 1	
		1 μ g/ml	10 μ g/ml		1 μ g/ml	10 μ g/ml
HDM sensitive (n = 17)	1,753.4 $\pm$ 415.1 (1,165.2)	577.9 $\pm$ 214.1 (75.9)	1,048.8 $\pm$ 320.4 (192.9)	652.3 $\pm$ 270.3 (182.0)	413.7 $\pm$ 181.5 (119.4)	804.3 $\pm$ 282.9 (339.4)
Non-atopic (n = 7)	900.8 $\pm$ 473.7 (267.3)	96.1 $\pm$ 77.7 (7.9)	331.5 $\pm$ 293.1 (17.9)	460.7 $\pm$ 268.5 (250.9)	45.7 $\pm$ 24.9 (15.8)	30.2 $\pm$ 18.8 (15.4)
HDM sensitive post-SIT ¹ (n = 10)	1,203.2 $\pm$ 328.5 (1,020.3)	769.0 $\pm$ 298.2 (258.1)	1,344.2 $\pm$ 425.2 (1,133.3)	349.0 $\pm$ 179.6 (92.2)	173.8 $\pm$ 104.4 (19.1)	363.3 $\pm$ 201.9 (29.2)

The results are the means $\pm$ SE (median in parentheses) of the IFN- γ levels in pg/ml measured by ELISA in culture supernatants on day 4. The lower limit of detection was 8 pg/ml.

¹ HDM-sensitive patients had been receiving immunotherapy with Dpt extracts for at least 12 months and had not had an injection within 3 weeks.

the manufacturer's instructions. The purity of the cell separation was tested by flow cytometry, and CD8-positive cells always constituted more than 96% of the cell population, with a mean level of $97.90 \pm 0.98\%$ (SD). Contamination of the CD8 cell preparation with CD4 cells was always less than 0.5%, with the mean contamination level being $0.20 \pm 0.17\%$ (SD). Cells were then resuspended in RPMI-1640 medium (ICN, USA) containing 5% human A serum. Assays were set up in triplicate in 96-well round-bottomed trays in a 200- μ l volume. Autologous PBMC were irradiated with 5,000 rad and used as APC.

Cytokine Measurement

IFN- γ was assayed by a two-monoclonal ELISA using commercially available, matched antibodies (Endogen, Woburn, Mass., USA) and standards (recombinant IFN- γ , Boehringer-Ingelheim, Germany). The lower limit of detection was 8 pg/ml. IL-4 and IL-5 were also both measured by two-monoclonal ELISA using commercial antibodies and standards. As the level of IFN- γ production from control cultures varied substantially, to allow comparison between patients in the peptide experiments, the cytokine responses to Der p 1 or the peptides were expressed as a stimulation index. Results of ≥ 5 were considered positive. In control or medium-alone cultures where IFN- γ levels were undetectable, a value of 8 pg/ml was assigned to the culture to allow calculation of the ratio.

Antigens and Peptides

Native Der p 1 was purified by affinity chromatography from spent mite medium. Peptides were synthesized by Chiron Mimotopes (Melbourne, Australia) as a DKP PepSet. Peptides were 19 amino acids in length and overlapped each other by 14 residues, apart from peptides 1–18 and 205–222. As the number of CD8 cells obtained from each patient was limited, for most experiments, the peptides were pooled into consecutive groups of 3.

Results and Discussion

IFN- γ release was measured from allergen-stimulated CD8 T cells and initial experiments included those to optimize conditions regarding CD8 cell concentration, APC number and type, culture medium, length of culture and allergen concentration. As Der p 1 induced optimal responses at concentrations of either 1 or 10 μ g/ml, both concentrations were used. In experiments on the time course, maximal responses were evident at either day 4 or 7 and day 4 was selected as the culture time. CD8 cells did not produce IFN- γ in the absence of irradiated PBMC for APC. Assays of supernatants from CD8 cultures for IL-4 and IL-5 failed to detect any consistent responses.

IFN- γ production by CD8 T cells from 17 Dpt-sensitive patients was compared to responses with cells from 10 patients who had received HDM SIT and another group of 7 who were non-atopic. Means and median results of 4-day assays of both CD8 cells and PBMC are presented in table 1. When PHA was used as a stimulus, there was no significant difference in IFN- γ production between any of the clinical groups for either PBMC or CD8 cells. For Der p 1-stimulated cultures of PBMC, significantly lower levels of IFN- γ were produced by PBMC of non-atopic subjects compared with atopic subjects ($p < 0.05$ for 1 and 10 μ g/ml), whereas similar production was evident from atopic non-SIT and post-SIT patients. With CD8 cells, production of IFN- γ was significantly lower in both the non-atopic and post-SIT groups, the levels being particularly low in the non-atopic controls (for 10 μ g/ml

Der p 1, $p = 0.006$ for atopic non-SIT versus non-atopics and $p < 0.05$ for atopic non-SIT versus atopic post-SIT).

The fact that IFN- γ release is high in atopic subjects and low in non-atopic individuals raises the possibility that the release and action of IFN- γ may be important in allergic inflammation. Indeed, through its ability to activate accessory cell function, stimulate IL-1, IL-6 and TNF- α release, induce adhesion molecule expression and support the recruitment and activation of inflammatory cells, IFN- γ could well be considered a proinflammatory cytokine that exacerbates allergic inflammation [8].

In patients receiving Dpt SIT there was also a significant reduction in the allergen-specific release of IFN- γ from CD8 T cells. The above finding of reduced IFN- γ release post-SIT argues against the role of an IFN- γ -producing 'suppressor' CD8 T cell as a mechanism of action of immunotherapy. We have also shown in previous research that there is a reduction in allergen-specific production of both IL-4 and IFN- γ from PBMC following SIT, arguing for the role of immune deviation as a mechanism of action of SIT [9]. From the above, we would propose that specific immunologic anergy or deletion could well be an important mechanism in SIT.

In the final phase of this project, 14 Dpt-sensitive patients and 6 non-atopic controls were tested for reactiv-

ity to a series of overlapping peptides of Der p 1, pooled into 14 consecutive groups of 3 peptides each. Eight of the 14 allergic patients reacted to one or more of the peptides with a stimulation index of at least 5. Two patients each reacted to 1, 2, 3 and 4 peptide groups. All the peptide groups were recognized by at least 1 of the patients and no particular group was recognized by more than 3 patients. As stated earlier, the major CD4 T cell epitopes on Der p 1 in both human and murine studies are located between amino acid positions 111 and 139. CD8 T cell epitopes in mice have also been predicted to be in the above regions and immunization experiments have confirmed strong reactivity using a Der p 1 peptide 111–139 [7]. Interestingly, in the epitope mapping experiments of Der p 1 reported here, reactive regions were present throughout the molecule with only 3 of 8 patients recognizing epitopes in this area.

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