

CD4⁺ T cells induced by virus-like particles expressing a major T cell epitope down-regulate IL-5 production in an ongoing immune response to Der p 1 independently of IFN- γ production

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Abstract

It has been previously demonstrated that hybrid Ty virus-like particles (VLP) prime effective CD8⁺ and CD4⁺ T cell responses. In this study, we investigated the effect of treating mice with Ty VLP carrying the immunodominant epitope of Der p 1 after sensitizing them to the group 1 allergen of the house dust mite *Dermatophagoides pteronyssinus* (Der p 1), under conditions that induce T_H2 immunity. We show that i.p. treatment with the hybrid VLP abrogated allergen-specific IL-5 production and reduced allergen-specific cell proliferation. This suppression of the response was mediated by CD4⁺ T cells and was not accompanied by an increase in IFN- γ production.

Introduction

Allergic diseases are characterized by the production of allergen-specific IgE, and the activation and local infiltration of mast cells, basophils and eosinophils. This response is orchestrated by allergen-specific T_H2 cells which secrete IL-4, IL-5, IL-10 and IL-13 (1–3).

The activation and infiltration of eosinophils is an important feature of allergy (reviewed in 4). Eosinophils release inflammatory mediators, such as peroxidase, cationic protein and major basic protein, which contribute directly to clinical symptoms. Eosinophilia occurs as a result of IL-5 production and high levels of eosinophils in the airways of asthmatic patients correlate with an increased amount of IL-5 mRNA (5). Furthermore, eosinophil infiltration into the lungs and the associated tissue damage is absent in IL-5-deficient mice after aeroallergen challenge (6). IL-5 induces the growth, differentiation and activation of eosinophils (7,8). It also induces the release of bone marrow eosinophils into the blood (9), enhances eosinophil survival (10) and increases the number of eosinophils infiltrating the tissues by enhancing eosinophil transendothelial migration (11).

A potential way of down-regulating IL-5 production is to switch the allergen-specific T_H2 response to a T_H1 response. Experiments in which this has been investigated have involved administration of the allergen with a carrier which induces T_H1-type cytokines (12), treatment with IL-12 (13) and immunization with IFN- γ DNA via the mucosal route (14). An alternative approach to down-regulating the allergic response is to render allergen-specific CD4⁺ T cells unresponsive. This has been investigated by administering high doses of peptides derived from an allergen via the respiratory mucosa, and induces profound and long-lasting tolerance (15–17).

The p1 protein of the yeast retrotransposon Ty can assemble into virus-like particles (VLP) containing ~300 molecules (18). This property has been exploited to produce an effective antigen presentation system. Fusion proteins comprising truncated p1 and antigen have been genetically engineered, and peptide sequences ranging from 1 to 43 kDa in size have been inserted, without disrupting particle formation

(19). This antigen presentation system induces effective cell-mediated immune responses (20,21) and the production of IFN- γ (21).

In this report, the effect of treatment with VLP carrying the immunodominant T cell epitope (residues 111–139) of Der p 1, a major allergen derived from the house dust mite *Dermatophagoides pteronyssinus*, was analysed in mice with established Der p 1-specific T_H2 responses. We demonstrate that IL-5 production was abrogated in these mice when the hybrid VLP were administered i.p. IL-5 down-regulation was accompanied by a decrease in allergen-specific T cell proliferation. This effect was mediated by allergen-specific CD4⁺ T cells, but was not the result of increased IFN- γ production.

Methods

Preparation of VLP

Hybrid Ty VLP carrying epitopes of Der p 1 were constructed and purified as previously described (18–20). The following VLP were produced: Der p 1 (111–139)–VLP, Der p 1 (113–127)–VLP and Der p 1 (111–119)–VLP. These hybrid VLP contain ~5% Der p 1 peptide. VLP lacking the p1 C-terminal Der p 1 peptide were used as a control.

Preparation of Der p 1 protein and peptide

The house dust mite allergen Der p 1 was purified from spent mite medium as previously described (16). The peptide sequence 111–139 was made by the Advanced Biotechnology Centre (London, UK).

Immunization of mice

Mice. Female C57BL/6 mice (Harlan Olac, Bicester, UK) were housed in the Central Biomedical Services Unit, Department of Biology, Imperial College. They were aged 6 weeks at the start of the experiment.

Sensitization of mice to Der p 1. The Der p 1–alum complex was made by mixing 2 μ g Der p 1 with 4 mg alum in a total volume of 100 μ l in saline per mouse and incubating this on a stirrer for 20 min at room temperature. The mice were immunized s.c. on day 0 with the Der p 1–alum complex. They were boosted with the same dose i.p. on day 21.

Treatment with the hybrid VLP and controls. Mice were treated i.p. with 100 μ g Der p 1–VLP, 100 μ g control VLP or 5 μ g (111–139) peptide in a total volume of 200 μ l in saline.

Cell culture

Single-cell suspensions were prepared from spleens removed 14 days after immunization with the hybrid VLP or controls. The cells were cultured at 5×10^5 cells in 200 μ l/well in RPMI-1640 (Gibco, Paisley, UK) supplemented with 10% FCS (GlobePharm, Guildford, UK), 100 IU/ml penicillin/streptomycin (Gibco), 2 mM L-glutamine (Gibco) and 5×10^{-5} M 2-mercaptoethanol (Sigma, Poole, UK) in flat-bottom 96-well plates. Der p 1 protein and peptide were added to the culture at different concentrations as indicated.

Measurement of cytokines and proliferation

Cytokines. The cells were incubated for 72 h before supernatants were collected for measurement of cytokines. IL-5 and IFN- γ production was measured by specific ELISA. Briefly, ELISA plates were coated with 2 μ g/ml anti-IL-5 mAb (TRFK5; PharMingen, San Diego, CA) or 3 μ g/ml anti-IFN- γ mAb (AN-18; a kind gift from Dr A. Livingstone) diluted in carbonate–bicarbonate buffer (Sigma) and incubated at 4° C overnight. The plates were blocked with 1% BSA (Sigma) in PBS for 30 min at room temperature. The supernatants were added at 50 μ l/well and incubated for 2 h at room temperature. IL-5 standard (X63-IL-5 cell supernatant) and IFN- γ standard (Serotec, Oxford, UK) were used for quantification. The presence of bound cytokine was detected using 2 μ g/ml biotinylated anti-IL-5 mAb (TRFK4; PharMingen) or a 1/1000 dilution of biotinylated anti-IFN- γ mAb (R4-6A2; Dr A. Livingstone) in PBS containing 1% BSA (Sigma) and 0.05% Tween 20 (Sigma), and incubated for 1 h at room temperature. This was followed by the addition of streptavidin–alkaline phosphatase (Sigma) for 30 min at room temperature. The addition of 1 mg/ml of the substrate, p-nitrophenyl phosphate (Sigma), diluted in ethanolamine buffer (Don Whitley, Shipley, UK) led to the production of a coloured product 15 min later. The intensity was measured at 405 nm using an ELISA reader.

Proliferation. The cells were incubated for 66 h before being pulsed with 1 μ Ci [³H]thymidine (ICN, Basingstoke, UK) per well and then incubated for a further 6 h. The cells were harvested onto glass fibre filtermats (Wallac, Turku, Finland) using an automated harvester (Mach III M; Tomtec, Hamden, CT). The incorporated radioactivity was measured in a liquid scintillation counter (1450 Microbeta Trilux; Wallac). The results are expressed as c.p.m.

Statistical analysis

The Student's *t*-test was used to measure the statistical significance of the experimental data. A value of *P* < 0.05 was considered significant.

Results

IL-5 production is abrogated when mice are treated with (111–139)–VLP i.p.

The sequence (111–139) contains the immunodominant region of Der p 1 for H-2^b mice. Within this peptide is an MHC class I-binding epitope (111–119) (21) and an MHC class II-binding epitope (113–127) (22). Mice were primed and challenged with Der p 1 in alum in order to induce a T_H2-type response to Der p 1. We then analysed the effect of treating these mice with (111–139)–VLP on splenocyte IL-5 secretion in response to Der p 1 peptide and protein *in vitro*.

Peptide-specific IL-5 was secreted after treatment of Der p 1-sensitized mice with control VLP, Der p 1 peptide or in the absence of treatment (Fig. 1a). In contrast, no detectable IL-5 was produced after treatment with (111–139)–VLP (Fig. 1a). Similarly, the level of IL-5 secreted in response to *in vitro* re-stimulation with the whole Der p 1 protein was significantly less in the group treated with (111–139)–VLP i.p.

than the control VLP-treated or untreated group ($P < 0.05$), but was not lower than the peptide-treated group (Fig. 1b).

Peptide-specific T cell proliferation is reduced after treatment with (111–139)-VLP i.p.

T cell proliferation was measured in order to analyse the extent of splenocyte responsiveness to Der p 1 in Der p 1-sensitized mice treated with (111–139)-VLP. Proliferation in response to the peptide *in vitro* remained at background levels after i.p. immunization with (111–139)-VLP (Fig. 2a). Although peptide-specific proliferation was low in the untreated group, it was significantly higher than in the (111–139)-VLP-treated group in response to 0.02, 0.2 and 2 μ M peptide *in vitro* ($P < 0.05$) (Fig. 2a). There was also significantly more proliferation after treatment with Der p 1 peptide and control VLP i.p. ($P < 0.05$) (Fig. 2a). Nevertheless, proliferation induced by the intact Der p 1 protein after treatment with (111–139)-VLP i.p. was not significantly lower than in the controls (Fig. 2b).

The abrogation of Der p 1-specific IL-5 production after treatment with (111–139)-VLP i.p. is not associated with Der p 1-specific IFN- γ production

IFN- γ inhibits cytokine production by T_H2 cells and prevents their expansion (14,23). We, therefore, analysed whether or not the decrease in IL-5 production observed after i.p. treatment of sensitized mice with (111–139)-VLP was accompanied by an increase in IFN- γ expression. Nevertheless, the level of IFN- γ secreted in response to Der p 1 peptide and protein *in vitro* after treatment of mice with (111–139)-VLP i.p. is the same as the amount produced after the control treatments (Fig. 3a and b). The level of IFN- γ secreted by the (111–139)-VLP, control VLP and peptide-treated groups was higher than that produced by the untreated group in response to peptide, but not protein, *in vitro* (Fig. 3a and b).

CD4⁺ T cells mediate the decrease in IL-5 after treatment with (111–139)-VLP i.p.

To determine whether the decrease in IL-5 observed after i.p. treatment with (111–139)-VLP was mediated by CD4⁺ or CD8⁺ T cells, Der p 1-sensitized mice were treated with VLP carrying (113–127), the CD4⁺ T cell-restricted epitope; (111–119), the CD8⁺ T cell-restricted epitope (111–139); both epitopes or control VLP. As an additional control, one group of Der p 1-sensitized mice was left untreated. Significantly more IL-5 was secreted after treatment with control VLP or (111–119)-VLP than after treatment with (111–139)-VLP or (113–127)-VLP ($P < 0.05$) (Fig. 4). Indeed, IL-5 secretion in response to both Der p 1 peptide and protein was completely abrogated after treatment with the VLP carrying the CD4⁺ epitope of Der p 1, indicating that the decrease in IL-5 production after treatment with (111–139)-VLP i.p. was mediated by CD4⁺ T cells.

Discussion

The experiments reported here evaluated whether treatment of Der p 1-sensitized mice with VLP expressing the immunodominant T cell epitope of Der p 1 ((111–139)-VLP) leads to the down-regulation of Der p 1-specific IL-5 production and/

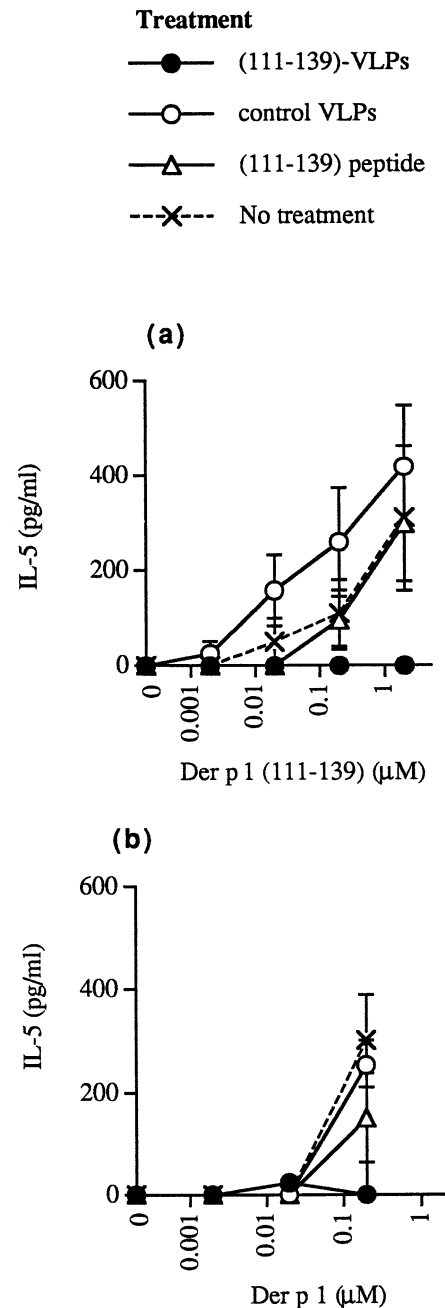


Fig. 1. Production is abrogated when Der p 1-sensitized mice are treated with (111–139)-VLP i.p. C57BL/6 mice were primed s.c. and boosted i.p. with der p 1 in alum. Twenty days later they were treated with (111–139)-VLP ($n = 4$), control VLP ($n = 4$) or (111–139) peptide ($n = 5$), or left untreated ($n = 3$). Fourteen days later the spleens were removed. The splenocytes were cultured *in vitro* with Der p 1 peptide (a) or protein (b) for 72 h before collection of supernatants for the measurement of IL-5. These results are representative of repeat experiments. Mean $\pm$ SEM is shown.

or the induction of IFN- γ . Mice were primed and boosted with Der p 1 complexed to alum to induce an antigen-specific T_H2 -type response. The effect on allergen-specific IL-5 and IFN- γ secretion of treating these mice i.p. with VLP carrying the dominant epitope of Der p 1 was then analysed.

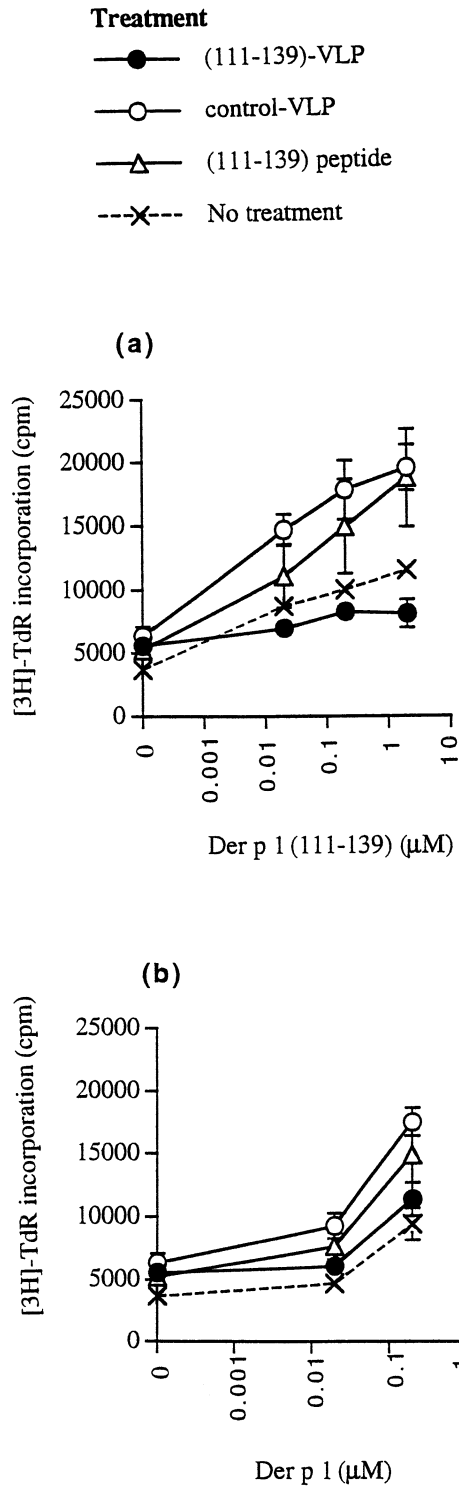


Fig. 2. Peptide-specific T cell proliferation is reduced after treatment of Der p 1-sensitized mice with (111-139)-VLP i.p. Mice sensitized with Der p 1 in alum (as in Fig. 1) were treated i.p. with (111-139)-VLP ($n = 4$), control VLP ($n = 4$) or (111-139) peptide ($n = 5$), or left untreated ($n = 3$). Fourteen days later the spleens were removed. The cells were cultured *in vitro* with Der p 1 peptide (a) or protein (b) for 72 h before measurement of proliferation. These results are representative of repeat experiments. Mean $\pm$ SEM is shown.

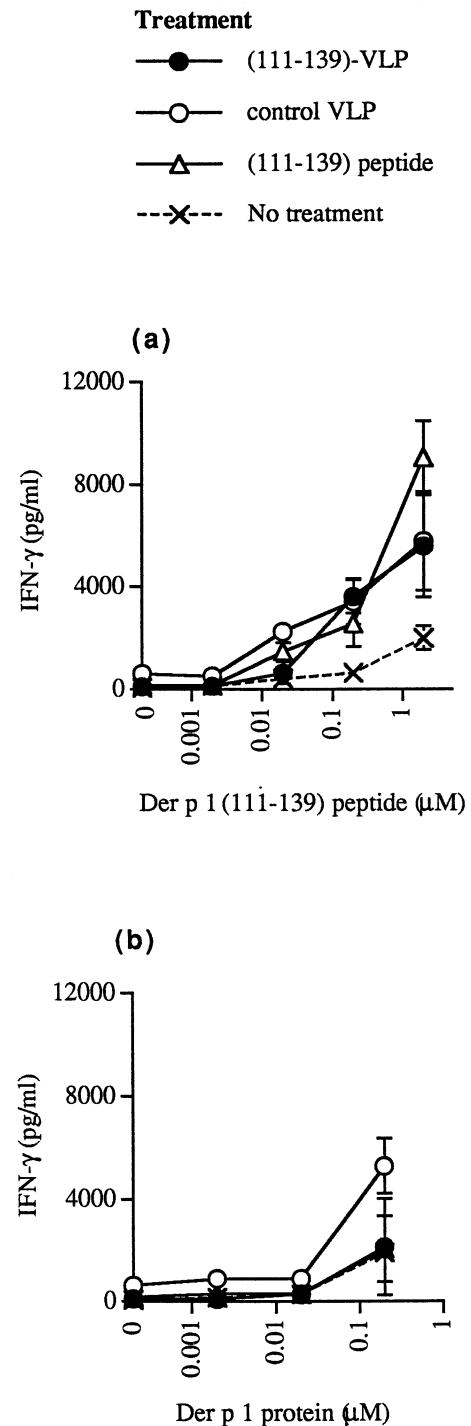


Fig. 3. IFN- γ production is not increased in Der p 1-sensitized mice after treatment with (111-139)-VLP, compared with the controls. Mice primed and boosted with Der p 1 in alum were treated with (111-139)-VLP ($n = 4$), control VLP ($n = 4$) or (111-139) peptide ($n = 5$), or left untreated ($n = 3$). Fourteen days later splenocytes were cultured *in vitro* with Der p 1 peptide (a) or protein (b) for 72 h before collection of supernatants for measurement IFN- γ . These results are representative of repeat experiments. Mean $\pm$ SEM is shown.

Priming and boosting mice with Der p 1 with alum leads to the generation of a high level of antigen-specific IL-5 secretion and T cell proliferation. The major finding reported here is that IL-5 production and T cell proliferation in response to the dominant epitope of Der p 1 was abrogated when Der p 1-sensitized mice were treated with (111–139)-VLP i.p. (Fig. 1). In contrast, splenocytes from Der p 1-sensitized mice treated with Der p 1 peptide or control VLP i.p. produced the same amount of IL-5 and proliferated to the same extent as cells from untreated mice (Fig. 1).

It is unlikely that the reduction in antigen-specific IL-5 secretion by T cells from (111–139)-VLP-treated mice occurred as a result of immune deviation of the response from T_H2 to T_H1 . The reduction in IL-5 production in the cultures was not accompanied by any increase in IFN- γ production (Fig. 3a and b), while peptide-specific proliferation by T cells from (111–139)-VLP-treated mice was markedly reduced compared with the controls (Fig. 2a). Rather, these data support the view that peptide-specific T cells are rendered anergic by treatment with (111–139)-VLP i.p.

The lack of effect of treatment with (111–139)-VLP i.p. on Der p 1-specific IFN- γ production indicates that only T_H2 , and not T_H1 cells, were rendered anergic. This is interesting in the light of previous reports that suggest that T_H1 cells are more susceptible to tolerance induction than T_H2 cells (24,25).

Several minor CD4⁺ T cell epitopes (residues 21–49, 78–100 and 197–212) have been mapped in Der p 1 (16,17). IL-5 production in response to the whole Der p 1 protein was diminished after i.p. treatment with VLP carrying only the immunodominant epitope of Der p 1 (Figs 1b and 4b). Thus, we came to the conclusion that there was no response to the minor epitopes. It may be that the epitope (111–139) is solely responsible for IL-5 production after sensitization to Der p1. Alternatively, linked suppression may have occurred. Linked suppression is the capacity to abrogate T cell responses to all epitopes in an antigen by treating with a single peptide and has been described in several models of peripheral tolerance (17,26,27).

The observed unresponsiveness may have occurred as a result of a direct toxic effect of the (111–139)-VLP on the Der p 1-reactive T cells, but experiments demonstrating an effective *in vitro* response to VLP suggests that this is unlikely (20). Alternatively, the (111–139)-VLP may have activated a regulatory subset of CD4⁺ T cells that modulates the function of Der p 1-reactive T cells through the secretion of inhibitory cytokines, such as IL-10 or transforming growth factor- β . This has been demonstrated in several other systems, such as experimental allergic encephalitis (28,29) and inflammatory bowel disease (30).

The induction of peptide-specific CD8⁺ T cells may be an effective way by which to down-regulate T_H2 cytokine-mediated responses (31). VLP carrying the H-2^D-restricted epitope, residues 111–119 of Der p 1, induce effective CD8⁺ cytotoxic T cell responses (21). We therefore analysed whether CD8⁺ or CD4⁺ T cells mediated the down-regulation in IL-5 production observed after i.p. immunization with (111–139)-VLP. Unexpectedly, our results suggest that CD8⁺ T cells were not involved (Fig. 4). A previous report studying the effect of inhaled protein revealed that down-regulation of IgE can occur in the absence of CD8⁺ T cells (32). CD8⁺ T cells

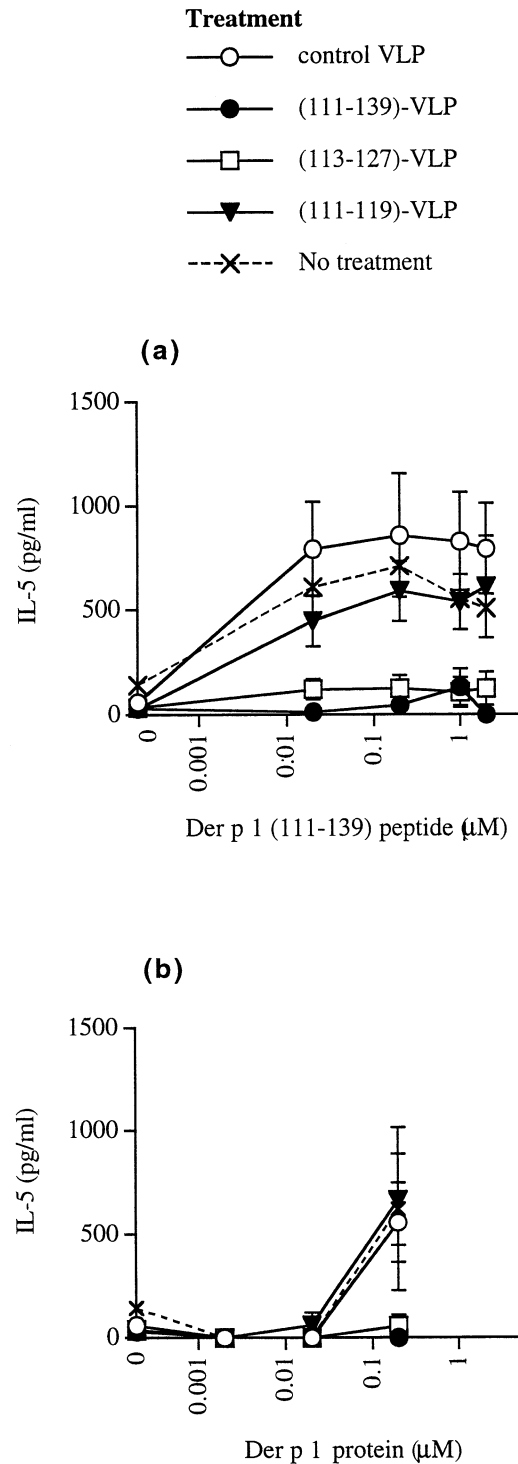


Fig. 4. The decrease in IL-5 production after treatment of Der p 1-sensitized mice with (111–139)-VLP is mediated by CD4⁺ T cells. Mice sensitized with Der p 1 in alum were treated i.p. with control VLP ($n = 4$), (111–139)-VLP ($n = 4$), (113–127)-VLP ($n = 4$) (111–119)-VLP ($n = 4$), or left untreated ($n = 3$). Fourteen days later splenocytes were cultured *in vitro* with Der p 1 peptide (a) or protein (b) for 72 h before collection of supernatants for measurement of IL-5. Mean $\pm$ SEM is shown.

were also not involved in a model of tolerance induced by a high dose of nasally administered peptide (33). Therefore, CD8⁺ T cells are involved in suppression of immune responses in some systems, but regulatory CD4⁺ T cells mediate non-responsiveness in others.

There is a need for more effective therapy for allergies in humans. Current allergen immunotherapy involves repeated immunizations with the native allergen and although it is often effective in reducing clinical symptoms, the risk of anaphylaxis is significant (35). VLP are safe in humans (36), and here we have demonstrated that VLP carrying the dominant epitope of Der p 1 are effective in abrogating allergen-specific IL-5 production after just one dose, and to the intact antigen. IL-5 is necessary for the generation of tissue eosinophilia and is thus an important target in the therapy of allergic disease. Therefore, the potential of VLP carrying epitopes of Der p 1 in the therapy of allergies warrants further investigation.

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Abbreviations

i.n. intranasal
VLP virus-like particle

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