

Scavenger receptor-specific allergen delivery elicits IFN- γ -dominated immunity and directs established T_H2-dominated responses to a nonallergic phenotype

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Background: Immunotherapeutic approaches to allergy consist of reliably changing allergen-specific T_H2 immunity associated with secretion of IL-4, IL-5, and IL-13, along with IgE antibodies in atopic individuals to T_H1 immunity. Our earlier data show that targeting of protein antigens to antigen-presenting cells (APCs), such as macrophages, by means of scavenger receptors (SRs) results in a pronounced T_H1 immunity. Here we demonstrate a novel experimental approach for the conversion of T_H2 immunity to T_H1 immunity by using SR delivery of allergens.

Objectives: We sought to show that targeting of allergens by means of SRs to APCs triggers T_H1 immunity and that an established T_H2 immunity to the Der p 1-immunodominant peptide 111-139 (p1, 111-139) can be modulated to a nonallergic T_H1 phenotype.

Methods: Analysis of the T cell-derived cytokines IL-4, IL-5, IL-13, and IFN- γ in response to p1, 111-139 in C57BL/6 mice 7 to 42 days after immunization, measurement of specific antibody responses, eosinophilic infiltrate, and skin hypersensitivity in response to allergen challenge constitute the parameters of *in vivo* immunity.

Results: We show that p1, 111-139, when delivered to APCs by means of SR, elicits a T_H1-dominant immunity. If it is delivered to APCs either after chemical coupling to SR ligands or by means of mere coadsorption on alum in the presence of an SR ligand, the established T_H2 immunity can be modified to T_H1 immunity.

Conclusions: SR-mediated delivery of allergens has immunotherapeutic potential that may be usable in atopic individuals. (*J Allergy Clin Immunol* 2002;109:321-8.)

Key words: Allergy, T_H1/T_H2, immunotherapy, scavenger receptor, immunomodulator, eosinophils, skin hypersensitivity

Some of the pathology responsible for allergic symptoms in atopic individuals is mediated by CD4 T cells, which produce a variety of cytokines and help B cells in producing high-affinity antibodies. The activation of naive CD4 T cells requires the recognition of both their target peptide MHC class II complexes and costimulatory signals from antigen-presenting cells (APCs). Responding CD4 T cells can be committed either to secreting T_H1 cytokines, such as IFN- γ and TNF- β , or T_H2 cytokines, such as IL-4, IL-5, and IL-13,^{1,2} whereas some maintain a T_H0 phenotype.^{3,4} Many factors, including accessory signals, cytokines, and the density and duration of peptide-MHC complexes presented by the APCs, contribute to differential commitment of CD4 T cells.⁵⁻⁸

We have been working on the ability of scavenger receptors (SRs), which are present on macrophages, dendritic cells, and B cells, to deliver antigens and characterizing immune responses after such a delivery.^{8,9} SRs are known to play a significant role in the initiation and modulation of atherosclerotic process by binding to oxidized low-density lipoprotein.¹⁰ In addition to low-density lipoprotein, many other protein and nonprotein ligands are reported for SRs, and some of them include maleylated proteins, polyguanylic acid (polyG), and fucoidin.^{9,11} We have shown that the delivery of protein antigens to APCs through SRs results in an enhancement of immunogenicity, as well as a preferential commitment of responding T cells to T_H1 cytokines, probably as a result of increased peptide-MHC complex availability rather than any modification of cell-associated costimulatory signals, such as CD80/86 or a known T_H1-triggering cytokine, IL-12.^{8,9}

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Abbreviations used

APC:	Antigen-presenting cell
HDM:	House dust mite
OVA:	Ovalbumin
p1, 111-139:	Der p 1-immunodominant peptide 111-139
PLL:	Poly-L-lysine
polyC:	Polycytidylic acid
polyG:	Polyguanylic acid
SR:	Scavenger receptor

The ability of previously primed and responding T_H2 or T_H1 cells to alter their cytokine-response profiles qualitatively is still controversial. Various reports say that T_H0 cells can be manipulated more easily, whereas stably committed T-cell clones can not,^{12,13} possibly because of irreversible silencing in the relevant cytokine genes.¹⁴⁻¹⁶ Altered peptide ligands may be a useful tool in achieving a change in the cytokine profile.¹⁷⁻¹⁹

The ability to modulate the cytokine profile of an ongoing primed T-cell response is potentially useful in the treatment of a range of clinical situations in which alteration of the T_H1/T_H2 balance is required. Allergen vaccination aims to achieve this by inhibiting T_H2 cytokine production, promoting the T_H1 components of responses, or both, thereby inducing nonallergic protective immunity. Most attempts at achieving this have been empirical thus far and have had variable results. The house dust mite (HDM) *Dermaphagoides pteronyssinus* is a major source of aeroallergens causing respiratory symptoms in susceptible individuals. The group 1 and group 2 allergens of HDM, Der p 1 and Der p 2, respectively, have been analyzed extensively for T- and B-cell epitopes in human and murine systems.²⁰⁻²² For the Der p 1 allergen, the immunodominant T-cell epitope in H-2^b mice has been mapped within residues 111 to 139.²³ We have investigated whether SR-specific delivery of the Der p 1-immunodominant peptide 111-139 (p1, 111-139) in vivo is able to modulate the cytokine profile of anti-p1, 111-139 immunity, and we demonstrate here that immunization with SR-directed p1, 111-139 not only induces IFN- γ -dominated primary immune responses but also downregulates established ongoing T_H2 responses.

METHODS

Mice and immunization

C57BL/6 mice obtained from the Jackson Laboratory and bred in the Small Animal Facility of the National Institute of Immunology were used throughout the experiment. Mice (5-8 per group) were immunized intraperitoneally or subcutaneously with 1 or 2 doses of various antigens in PBS or adsorbed on alum (Alhydrogel, Superfos) as indicated. For the T-cell assays, mice were euthanized as indicated after immunization, and single-cell splenic suspensions were used. For estimating antibody concentrations, mice were bled from the retro-orbital venous plexus after achievement of inhalational anesthesia. All animal experimentation was done with the approval of the Institutional Animal Ethics Committee.

Chemical modification and coupling of immunogens

Conalbumin, ovalbumin (OVA), or poly-L-lysine (PLL; Sigma Chemical Co) were maleylated, as described previously,^{8,9} with maleic anhydride (Sigma) at alkaline pH, followed by extensive dialysis against PBS. The extent of maleylation was measured by estimating the loss of free ϵ -amino groups of the protein by using trinitrobenzene sulphonic acid (Sigma).⁸ A synthetic peptide from the Der p 1 sequence p1, 111-139 was coupled with glutaraldehyde to either native proteins or to their maleylated forms. Alternatively, p1, 111-139 was chemically coupled to the 3'-thiolated decameric oligonucleotides polyG or polycytidylic acid (polyC; Biosynthesis Inc). Essentially, peptide was conjugated with sulphosuccinimidyl 6-[3'-2-(pyridyldithio)propionamido] hexanoate (Sulpho-LC-SPDP, Sigma), after which it was coupled to 3'-thiolated oligos, as previously described.²⁴ Either PLL or maleylated PLL were coadsorbed with p1, 111-139 at equal concentrations on alum for immunization where appropriate. All the carriers used for p1, 111-139 targeting to SR (ie, PolyG, maleylated conalbumin, and maleylated PLL) inhibit activation of OVA-specific T-cell hybridoma in a dose-dependent manner, whereas polyC, conalbumin, and PLL do not, demonstrating their ability to compete for SR binding (data not shown).

Estimation of antibody levels

Sera from immunized mice were used for estimation of antigen-specific IgG2a and IgE, as described previously,^{8,25} with p1, 111-139-coated plates, followed by serum dilutions. The specific binding was detected with goat anti-mouse IgG2a (Sigma) or biotinylated rat anti-mouse IgE (PharMingen) as secondary reagents, followed by rabbit anti-goat Ig peroxidase or streptavidin-coupled alkaline phosphatase. Antibody amounts in sera were quantitated by comparison with known standards assayed in parallel. Results are expressed as means $\pm$ SEM for each group.

For estimation of total serum IgE levels, monoclonal anti-IgE (PharMingen) was used as the coating reagent. Bound IgE was detected with polyclonal anti-mouse IgE biotin (PharMingen), followed by avidin-peroxidase (Vector, Burlingame) and quantitated by means of comparison with a known standard.

Lymphocyte proliferation and cytokine assays

Splenic cells from immunized mice were cultured in the presence of titrated amounts of antigens in 200- μ L cultures in 96-well tissue-culture plates with Click's medium (Irvine Scientific, Santa Ana, Calif) containing 10% FBS (Biological Industries), glutamine, and antibiotics. After incubation, cultures were pulsed with tritiated thymidine (New England Nuclear), harvested, and counted by means of scintillation spectroscopy (Beta-plate, LKB-Pharmacia). Counts from triplicate cultures are expressed as means $\pm$ SEM. Counts in the absence of antigen varied from 800 to 3000 in different experiments. For cytokine measurements in T-cell activation assays, culture supernatants were harvested at 60 hours and used for the estimation of IL-4, IL-5, IL-10, IL-13, and IFN- γ by using commercially available enzyme immunoassay systems (R&D Systems). In brief, anti-cytokine capture antibodies were coated onto plates, followed by culture supernatants and then by peroxidase-labeled detecting antibodies. Known recombinant standard cytokines were run in parallel in each assay to quantitate the cytokine amounts present. The cytokine ratio values are means $\pm$ SEM of individual ratios obtained at each recall antigen dose on the basis of concentrations calculated from reference standards.

Peritoneal challenge and evaluation of exudate cells

For experiments aimed at evaluating allergic responses *in vivo*,²⁶ mice received p1, 111-139 (100 µg/mouse) coadsorbed with PLL on alum subcutaneously on day 0, followed by p1, 111-139 (100 µg/mouse) coadsorbed on alum with either PLL or maleylated PLL on day 8. On day 15, mice were challenged with 10 µg of p1, 111-139 per mouse intraperitoneally in PBS. Peritoneal washout cells were harvested 6 hours after challenge, and total and differential counting of leukocyte subpopulations was done. A minimum of 400 cells from Leishman-stained smears were counted for differential counts. Results are expressed as total numbers of cells (mean ± SEM, *n* = 3).

Measurement of allergic skin hypersensitivity reaction

For measurement of acute skin hypersensitivity reaction, a modification of a published protocol²⁷ was used. Mice were immunized as described above for the allergic peritonitis model. A challenge dose of 10 µg of p1, 111-139 was given intradermally in 10 µL of PBS containing trypan blue to facilitate measurement of the skin reaction at 0, 0.5, 1, 2, and 7 hours after injection. The fold increase over the 0-hour reading (mean ± SEM, *n* = 3) is shown as an indicator of the magnitude of the skin hypersensitivity reaction.

Statistical analysis

Statistical analysis was done with the Student *t* test wherever appropriate. The data are representative of between 2 and 7 experiments.

RESULTS

Immune responses to p1, 111-139 after SR-directed immunization

We have previously reported that immunization of mice with SR-binding maleylated proteins induces IFN-γ-dominated immune responses.⁸ Immune responses to the immunodominant epitope²³ of the Der p 1 allergen of HDM, p1, 111-139, in H-2^b mice (C57BL/6) were studied to extend these results to allergen immunomodulation. T-cell cytokine responses generated by immunization with p1, 111-139 on alum were compared with those generated by p1, 111-139-conalbumin or p1, 111-139 coupled to maleylated conalbumin in PBS 8 days after immunization. Splenic cells from all groups mounted marked proliferative responses *in vitro* against p1, 111-139 (Fig 1, A). In addition, the mice immunized with p1, 111-139-conalbumin and p1, 111-139-maleylated conalbumin also responded strongly to conalbumin and maleylated conalbumin (data not shown). T cells from mice immunized with either p1, 111-139-alum or p1, 111-139-conalbumin secreted high levels of IL-5 and IL-10 and relatively low levels of IFN-γ, whereas T cells from p1, 111-139-maleylated conalbumin-immune mice secreted little IL-5 or IL-10 but high levels of IFN-γ (Fig 1, B-D). As a result, the cytokine ratios indicating the T_H1/T_H2 balances achieved were very different in p1, 111-139-maleylated conalbumin-immune mice compared with conalbumin-immune mice (Fig 1, E). Thus SR-specific delivery of p1, 111-139 during primary immunization by coupling it to maleylated conalbumin results in a T_H1-dominated anti-p1, 111-139 immune response. The

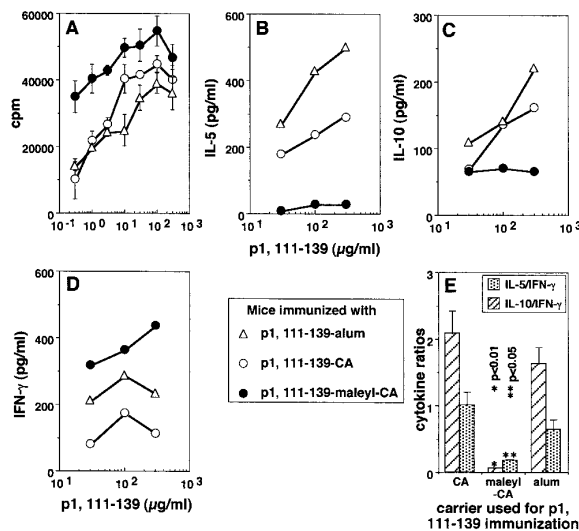


FIG 1. Primary immunization with p1, 111-139 coupled to maleylated conalbumin (p1, 111-139-maleyl-CA) results in IFN-γ-dominant response against the peptide. Responses of splenocytes from immunized mice to titrating amounts of p1, 111-139 are shown as proliferation (A) and culture supernatant levels of IL-5 (B), IL-10 (C), and IFN-γ (D), as well as respective cytokine ratios (E).

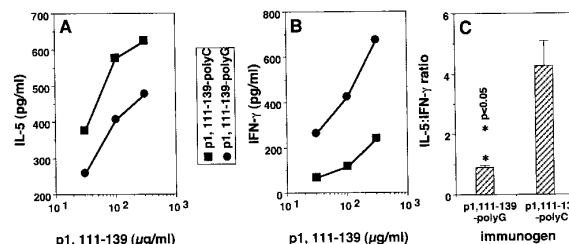


FIG 2. IFN-γ-dominant response to SR-directed p1, 111-139 immunization is independent of carrier epitopes. Splenocytes from immunized mice were stimulated with p1, 111-139. Culture supernatants were analyzed for levels of IL-5 (A) and IFN-γ (B). C shows the resultant cytokine ratios.

conalbumin-specific cytokine responses also followed a similar trend (data not shown), as reported earlier.⁸

The above data establish that even an epitope from a strong T_H2 immunogen, such as Der p 1, could lead to T_H1-dominated responses if delivered to SRs, despite the presence of competing carrier-protein epitopes. To avoid competition for immune response by carrier-protein epitopes, we coupled p1, 111-139 to a known nonpeptidic SR ligand, decameric polyG, or to a control oligonucleotide, polyC, that cannot bind SRs.¹¹ Immunization of mice with p1, 111-139-polyG in PBS generated IFN-γ-dominated responses (Fig 2, B). In contrast, p1, 111-139-polyC immunization resulted in greater IL-5 production instead (Fig 2, A), resulting in lower IL-5/IFN-γ ratios in the p1, 111-139-polyG-immune mice (Fig 2, C). As expected, neither T-cell responses nor antibodies directed against polyG or polyC were detectable (data not shown).

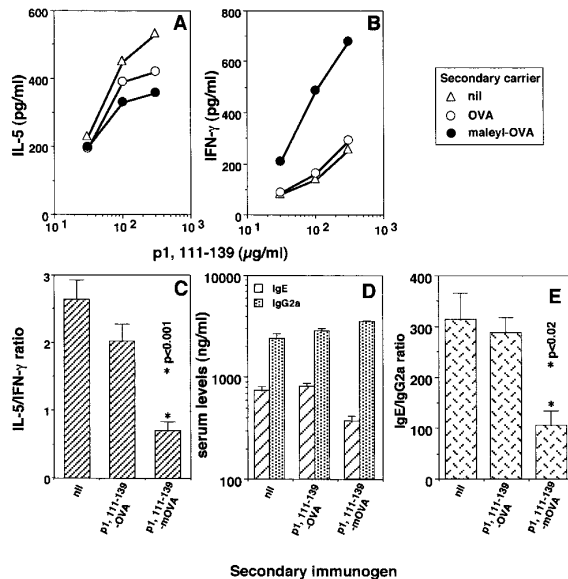


FIG 3. Cytokine profiles shift toward T_H1 , with a decrease in IgE during secondary immunization with SR-directed p1, 111-139. Anti-p1, 111-139 cytokine responses of splenocytes from p1, 111-139-alum-immune mice given boosts as indicated. Levels of IL-5 (A) and IFN- γ (B) and cytokine ratios (C) are shown. Total IgE level, peptide-specific IgG2a levels (D), and their relative proportion as ratios (E) are shown.

Effect of secondary immunization with p1, 111-139 coupled to SR ligands on ongoing p1, 111-139-specific responses

Our data thus far demonstrate that SR-directed delivery, even of epitopes from allergens, induces T_H1 -dominated responses. However, these data do not address the issue of modulating the cytokine profiles of ongoing responses, an issue central to the immunotherapy of allergic disorders. We therefore examined the effects of secondary immunization with SR-directed immunogens on cytokine profiles in mice after allergen exposure.

Mice primed intraperitoneally with p1, 111-139-alum were given either p1, 111-139-OVA or p1, 111-139-maleylated OVA intraperitoneally in PBS or PBS alone after 2 week. Cytokine patterns of splenic cells responding in vitro to p1, 111-139 were determined 7 days after secondary immunization. There was a small but consistent reduction in IL-5 levels (Fig 3, A) and a significant increase in IFN- γ levels (Fig 3, B) secreted by T cells from mice boosted with p1, 111-139-maleylated OVA. The IL-5/IFN- γ ratio did not change significantly after secondary immunization with p1, 111-139-OVA; however, it did after administration of p1, 111-139-maleylated OVA (Fig 3, C).

IgE antibodies are known to be crucial in the pathology of allergy, and hence we measured total IgE and p1, 111-139-specific IgG2a levels as indirect markers of T_H2 and T_H1 immunity, respectively. An increase in p1, 111-139-specific IgG2a was accompanied by a reduction

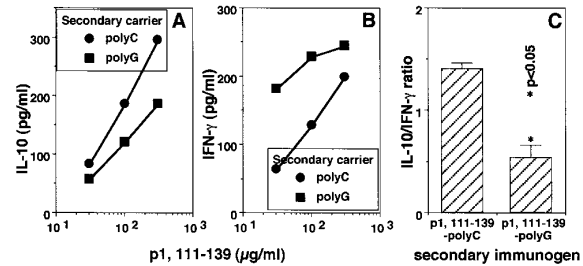


FIG 4. SR-directed secondary immunization with p1, 111-139 redirecTs T_H1/T_H2 profiles, even with nonprotein SR ligand as the carrier. Mice immunized with p1, 111-139-polyC were boosted as indicated, and splenocytes were stimulated with p1, 111-139. Culture supernatants were analyzed for IL-10 (A) and IFN- γ (B) levels, and the IL-10/IFN- γ ratios (C) were calculated.

in total IgE levels in mice boosted with p1, 111-139-maleylated OVA (Fig 3, D), resulting in significantly lower IgE/IgG2a ratios after the p1, 111-139-maleylated OVA booster (Fig 3, E).

Similarly, either polyG or polyC was used as a carrier for p1, 111-139 in secondary immunization. Mice immunized with p1, 111-139-polyC in PBS 1 week earlier were given either p1, 111-139-polyC or p1, 111-139-polyG, and T-cell cytokine responses were assayed a week later. Once again, a similar shift in the immune response from T_H2 to T_H1 dominance was observed, with secondary p1, 111-139-polyG immunization skewing the ongoing T_H2 -dominated response toward IFN- γ (Fig 4).

SR-specific delivery of p1, 111-139 without chemical coupling in the presence of the standard adjuvant alum

The data thus far suggest that SR-specific secondary immunization could reverse ongoing allergic immune responses, even without using any conventional adjuvant. In the clinical situation alum is one of the few adjuvants acceptable for human use. Therefore the immunotherapeutic potential of the SR-specific delivery of alum-adsorbed p1, 111-139 was next analyzed. For immunization, p1, 111-139 was coadsorbed with either PLL or maleylated PLL on alum. Mice were immunized with p1, 111-139 PLL (300 μg of p1, 111-139/mouse). Secondary immunization, given 7 to 14 days later, consisted of p1, 111-139 coadsorbed on alum with either PLL or maleylated PLL. Seven days after the secondary immunization, splenic T cells were used in p1, 111-139-specific recall assays in vitro. T cells from mice boosted with p1, 111-139-maleylated PLL secreted only minimal levels of IL-5 (Fig 5, A) but high levels of IFN- γ (Fig 5, B) compared with the group boosted with p1, 111-139-PLL. Thus the simple expedient of coadsorbing the epitope on alum with a nonimmunogenic SR ligand was sufficient to shift the cytokine profile of the ongoing T-cell response to it away from the allergic T_H2 phenotype (Fig 5, C). The extent of a shift away from T_H2 immunity was also ana-

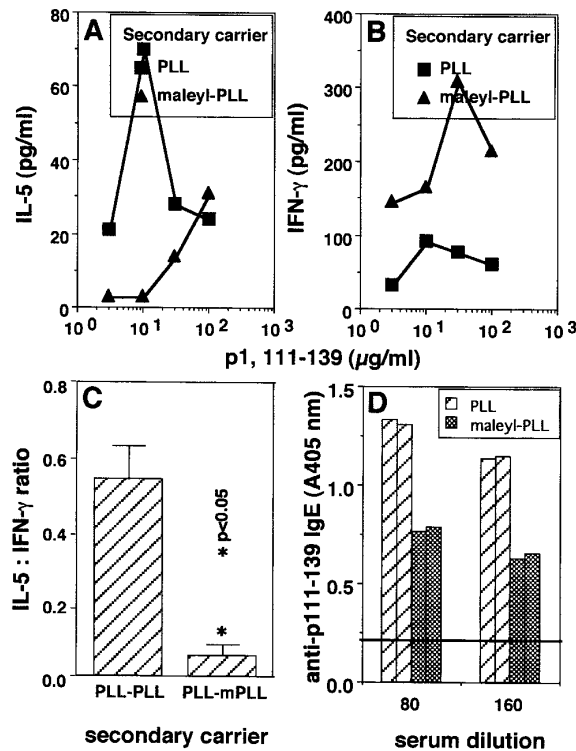


FIG 5. SR-directed delivery by coadsorption of p1, 111-139 with maleylated PLL on alum is sufficient to change T_H1/T_H2 balances. Mice primed with p1, 111-139-PLL-alum were boosted with p1, 111-139 coadsorbed with alum, as shown. Seven days later, splenocytes were stimulated *in vitro* with p1, 111-139. Levels of IL-5 (A) and IFN-γ (B) in culture supernatants and IL-5/IFN-γ ratio (C) are shown. D shows the p1, 111-139-specific IgE levels at indicated dilutions of sera pooled from 5 to 8 animals per group. Carrier for secondary immunization is indicated. The horizontal line shows background absorbance values.

lyzed for peptide-specific IgE responses. Levels of p1, 111-139-specific IgE were reproducibly lower in mice receiving secondary immunization with p1, 111-139-maleylated PLL than in mice receiving p1, 111-139-PLL as the secondary immunogen (Fig 5, D).

The cytokine profile of T cells was also evaluated 28 days after mice received a second dose of immunization. T cells from mice receiving p1, 111-139-maleylated PLL as a second dose produced higher levels of IFN-γ and lower levels of IL-4, IL-5, IL-10, and IL-13 compared with those receiving p1, 111-139-PLL as a secondary immunogen at day 7, as shown (Fig 6, left panels). At 28 days after secondary immunization, all responses were lower than those on day 7; however, the overall pattern remained the same (Fig 6, right panels).

Effect of secondary immunization with SR-targeted p1, 111-139 on allergic manifestations *in vivo*

The efficacy of the immunomodulatory approach was evaluated with *in vivo* models to assess its clinical utili-

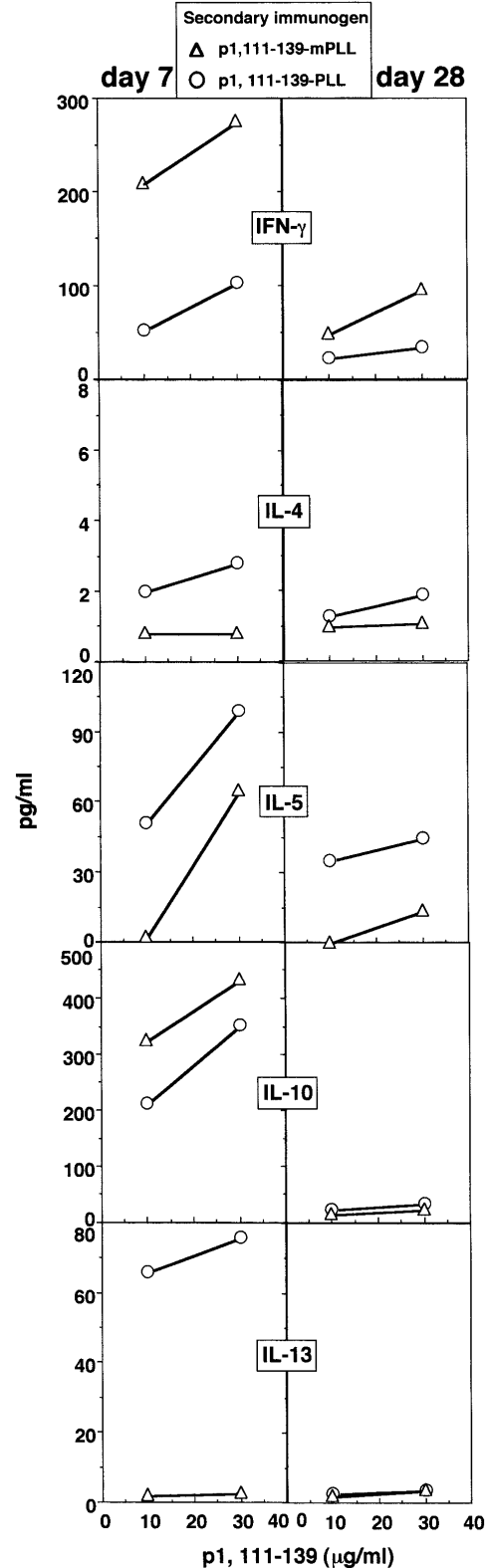


FIG 6. Levels of cytokines decline over time, but immune polarity is persistent over a 1-month period. Mice primed and boosted as in Fig 5 were followed until day 28, and cytokine levels in culture supernatants from splenic cultures analyzed on days 7 and 28 after the booster are shown.

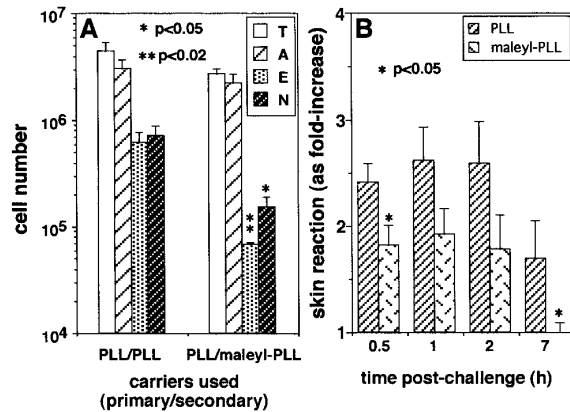


FIG 7. SR-directed delivery of p1, 111-139 with maleylated PLL on alum abrogates allergic manifestations in vivo. Mice primed with p1, 111-139-PLL-alum boosted with either p1, 111-139-PLL-alum (PLL/PLL) or p1, 111-139-maleylated PLL-alum (PLL/maleyl-PLL) were challenged a week later with p1, 111-139 in PBS administered either intraperitoneally (**A**) or subcutaneously (**B**). **A** shows the numbers of total cells (T), agranulocytes (A), eosinophils (E), and neutrophils (N) harvested from the peritoneum 6 hours after intraperitoneal challenge. **B** shows the fold increase in diameter above the 0-hour value after subcutaneous challenge. Carriers for secondary immunization are indicated.

ty. For analyzing the effect on eosinophilic infiltrate in the allergic peritonitis model,²⁶ mice were immunized with p1, 111-139-PLL-alum, followed a week later with either p1, 111-139-PLL-alum or p1, 111-139-maleylated PLL-alum. One week after the secondary immunization, the mice were challenged with p1, 111-139 intraperitoneally, and the peritoneal cellular infiltrate was characterized 6 hours after challenge. The numbers of eosinophils and neutrophils extravasating in mice receiving p1, 111-139-maleylated PLL-alum as a secondary immunogen were far lower than those for mice receiving p1, 111-139-PLL-alum (Fig 7, A).

For analyzing allergic skin response, an immunization regimen similar to the one mentioned for allergic peritonitis was used, after which a subcutaneous challenge was given with p1, 111-139 in PBS. The magnitude of the resultant skin reaction was monitored over a 7-hour period. Mice primed, as well as boosted, with p1, 111-139-PLL showed a larger skin hypersensitivity response than mice receiving p1, 111-139-maleylated PLL as a secondary immunogen. Although the skin reaction was still above background levels at the end of 7 hours in the former group, it had receded completely in the latter (Fig 7, B), establishing that allergic skin reactions in vivo in allergen-immunized mice can also be abrogated by the use of SR-specific epitope delivery in vivo.

DISCUSSION

Immunotherapy is one of the major approaches explored for effective control of allergy in human subjects.²⁸ Here we report a new experimental approach to change estab-

lished T_H2 immunity to the HDM-derived immunodominant peptide p1, 111-139 to T_H1 immunity. In clinical situations it is common to observe a mixed response of either T_H0/T_H1 or T_H0/T_H2 type to the antigens,²⁹⁻³¹ which is unlike experimental situations, in which clear polar T_H1 or T_H2 responses can be observed.^{2,5,6} Hence we have looked at the resultant cytokine balance as an indicator in an immune response to p1, 111-139.

In our model system with use of the dominant epitope of Der p 1 HDM allergen,³² we observed that p1, 111-139 can evoke a T_H1-dominated response if delivered to SRs in vivo, suggesting that SR-specific delivery could overcome the strong T_H2 tendencies of allergens (Fig 1). Furthermore, the T_H1-skewed immune responses to carrier-protein epitopes were not necessary for p1, 111-139 to evoke a T_H1 response because coupling to a nonimmunogenic SR ligand, polyG, also yielded similar cytokine profiles (Fig 2). Success of protein, as well as nonprotein, ligands of SRs as carriers widens the scope of the utility of this approach.

The delivery of p1, 111-139 to SRs after allergen exposure successfully redirects the immune response toward the T_H1 pole and away from T_H2 immunity. This can be achieved either by using an immunogenic protein carrier, such as OVA (Fig 3), or a nonprotein carrier, such as polyG (Fig 4). We have observed that coating alum with SR ligands allows its delivery to APCs, with a resultant enhancement in immunogenicity and a skewing of the T_H1/T_H2 balance toward IFN- γ for a variety of protein antigens (data not shown). Because the immune responses generated by SR-specific delivery of alum-adsorbed antigens are of much longer duration than those induced by maleylated proteins in PBS (data not shown), we used this modality to convert an allergenic immunogen into triggering a protective response. PLL is nonimmunogenic by itself and can be used as a ligand for SR delivery very efficiently because potentially every lysine can be maleylated, thereby making it an efficient ligand for SRs. We have shown that simple coadsorption of the peptide on alum with maleylated PLL is sufficient to elicit T_H1-dominant responses and to redirect ongoing allergy (Figs 5 and 7), providing a versatile tool for allergen immunotherapeutic approaches. This approach provides a method for coadsorption of more than one peptide of interest along with maleylated PLL on alum to achieve redirection of the immune response, a point of relevance in clinical situations in which patients can be allergic to more than one allergen. Additionally, the immunomodulation achieved with one dose appears to persist, albeit weakly, for about a month after the secondary immunization (Fig 6). Although further boosters might be needed for longer persistence, the approach does show promise for therapeutic application.

The role of IgE in mediating allergy makes it essential to examine antibody responses in potential modalities of allergen vaccination. We observe that, even in the presence of ongoing T_H2 immune responses, SR-directed vaccination with an allergen-derived epitope brings about decreases in both T_H2 cytokines and IgE levels.

The reduction in IgE levels is seen both in total and p1, 111-139-specific IgE levels (Figs 3 and 5). Although p1, 111-139 immunization resulted in generating anti-Der p 1 IgG1 and IgG2a responses (data not shown), Der p 1-specific IgE was not detectable. Because all immunizations in the present system are done with the synthetic T-cell peptide epitope alone, it is not surprising that this peptide is not being recognized optimally in the conformationally altered state in the native molecule, rendering detection of low-level IgE technically difficult.

The test of the utility of an allergy-modifying intervention lies in its capacity to modulate allergic lesions in vivo. Skin sensitivity reactions are a hallmark of allergy, and eosinophilic infiltration at the site of lesions is associated with pathogenesis in allergic inflammation. SR-mediated delivery of p1, 111-139 also inhibits acute skin hypersensitivity responses²⁷ and inhibits eosinophil recruitment in allergic peritonitis in vivo (Fig 7), providing further evidence for its potential clinical utility. Although we have not been able to detect the eosinophil chemoattractant eotaxin in activated T-cell cultures (data not shown), it is known that IFN- γ has a negative regulatory effect on eotaxin production.³³ It is therefore possible that the increase in IFN- γ induced by SR-directed immunogen inhibits eotaxin induction and eosinophil recruitment in vivo.

The avidity of peptides for a given MHC molecule is known to influence the T_H1/T_H2 balance of responding T cells,^{7,8} with higher avidity associated with preferential T_H1 commitment of cells. SR-mediated delivery of antigens to APCs results in the generation of a higher density of peptide-MHC class II complexes, which persist for a longer period of time.⁸ Therefore it is possible that the increase in ligand density generated by antigen delivery to SRs promotes T_H1-dominant responses, even in experienced T-cell populations, and this may account for the redirection of ongoing T_H2 responses that we observed. The duration of time after priming during which this plasticity can still be evoked by high densities of peptide MHC class II complexes is a crucial issue for subsequent investigations.

In conclusion, we report here an approach to allergen vaccination on the basis of generating high densities of peptide-MHC class II complexes by delivering antigen to SR-bearing APCs, which results in reversal of T-cell T_H2 cytokine generation and IgE production, as well as reversal of allergic inflammation in vivo. The approach relies on simple coadsorption of allergen with a nonimmunogenic SR ligand on alum, providing wide potential clinical utility in all situations in which allergenic molecules can be identified.

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