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Threshold Signaling of Human Th0 Cells in Activation and Anergy: Modulation of Effector Function by Altered TCR Ligand

Adrienne Verhoeef* and Jonathan R. Lamb†

Molecular interactions between TCR and its natural ligand, in the presence of costimulatory signals, elicit T cell effector functions, whereas subtle changes in the structure of antigenic peptides may induce only selected T cell effector function including anergy. In this study, we have investigated the immunological activity of an altered TCR ligand (p 2, 28–40A34,36) derived from the immunodominant T cell epitope of the group 2 allergen of house dust mite, in which residues at positions 34 and 36 were substituted by alanine. Elevated IFN-γ synthesis was induced by equimolar concentrations of the analogue compared with native peptide (p 2, 28–40) and was paralleled by increased down-regulation of cell surface CD3. IL-5 and IL-10 production exhibit the same sensitivity to both peptides, implying that the induction of T cell effector functions are not all proportional to TCR occupancy. Both native peptide and the analogue bound to MHC class II (DRB1*1101) molecules with similar affinities. Furthermore, p 2, 28–40A34,36 induced T cell anergy at lower concentrations than native peptide. During the induction of anergy, TGF-β production was comparable for both peptides, whereas IL-10 secretion was markedly increased but more so in response to p 2, 28–40A34,36. Membrane expression of costimulatory ligands CD80 and CD86 was similar for native peptide and p 2, 28–40A34,36 and increased in activation, whereas only CD86 was elevated during anergy. The modulation of T cell effector function with altered TCR ligands may have practical applications in reprogramming allergic inflammatory responses through the induction of T cell anergy and/or the promotion of Th1 cytokines. The Journal of Immunology, 2000, 164: 6034–6040.

Both the local microenvironment and characteristics of the ligands that T cells encounter influence the cytokines they produce, and, consequently, the qualitative nature of the immune response that is generated (1–3). In individuals with allergic disease, analysis of the phenotype of CD4+ T cells isolated from peripheral blood (4–6) and local sites of inflammation (7, 8) suggests that they belong to the Th2/Th0 subset. Their functional activity is characterized by cytokine response profiles dominated by the secretion of IL-4, IL-5, and IL-13, with only low amounts of IFN-γ detectable. The production of these Th2 cytokines promotes the synthesis of allergen-specific IgE and the recruitment of eosinophils, both of which mediate allergic inflammation (9, 10). In contrast, the allergen-specific T cell repertoire of nonallergic individuals comprises Th1/Th0 cells, and the production of Th1 cytokines is associated with “protective immunity” (11). These observations underline the premise that correcting the Th2/Th1 balance in allergic individuals may form the basis of successful allergen immunotherapy which, in principle, may be achieved by tolerizing Th2 cells or promoting Th1 cytokine-mediated immunity (12, 13).

Ligation of T cell Ag receptor (TCR) by antigenic peptides bound to MHC class II molecules, in conjunction with costimulatory signals provided by APCs, induces the effector functions of CD4+ T cells (14, 15). It is documented that qualitative and quantitative differences in Ag recognition may affect the affinity of interactions between TCRs and their ligands. This may arise from the introduction of subtle changes in the structure of antigenic peptides (16, 17). For example, stimulation of Th1 cells in vitro with an altered TCR ligand inhibited cytokine production and proliferation, while cytolytic activity was maintained (16). In a comparable study, it was reported that Th2 cells stimulated with peptide analogues retained their capacity to produce IL-4 and provide B cell help, but failed to expand (17). Differential effects of peptide analogues in vivo have also been described, particularly with regard to their influence on Th1/Th2 differentiation (18, 19).

Certain altered TCR ligands have dominant negative effects on T cell function. The stimulation of human CD4+ T cells with supraoptimal concentrations of wild-type peptide renders them unresponsive to antigenic restimulation (21, 22). Peptide analogues with partial agonist activity have been identified, which when presented by live APCs, induce anergy in both murine Th1 and Th2 cells (23). The immunoregulatory effects of altered TCR ligands with similar functional activities have been demonstrated in vivo, for example, in the reversal of experimental autoimmune encephalomyelitis (24).

The human peripheral T cell response to the group 2 allergen (Der p 2)3 of Dermatophagoides pteronyssinus (house dust mite, *Department of Biology, Imperial College of Science, Technology and Medicine, London, United Kingdom; and Respiratory Medicine Unit, Edinburgh University Medical School, Edinburgh, United Kingdom

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2 Address correspondence and reprint requests to Dr. Jonathan Lamb, Respiratory Medicine Unit, Rayne Laboratory, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG, U.K. E-mail address: j.r.lamb@ed.ac.uk

3 Abbreviations used in this paper: Der p 2, group 2 allergen of house dust mite Dermatophagoides pteronyssinus; HDN, house dust mite.
HDM) is directed primarily at two immunodominant regions, residues 28–40 (p 2, 28–40) and 101–129 (25–27). We have previously reported that two peptide analogues of p 2, 28–40 with single alanine substitutions at positions 34 and 36 enhanced IFN-γ but not IL-4 production by a clone of specific TH0 cells (28). Here, that study is extended, and the effects of a peptide analogue with a double alanine substitution at residues 34 and 36 (p 2, 28–40A34,36) on clonal expansion, cytokine production, and modulation of the surface phenotype during activation and the induction of anergy are reported. Our results indicate that the analogue has increased affinity for the TCR and behaves as a “superagonist” for selected effector functions.

Materials and Methods

Antigens

The Der p 2 peptides, p 2, 28–40 (IHRGTKPFQLEAV) and p 2, 28–40A34,36 (IHRGKAFALEAV) were purchased from Genosys (Cambridge, U.K.). The peptides were tested for nonspecific immunosuppressive and mitogenic activity on cloned T cells reactive with influenza virus hemagglutinin. No modulation of normal effector function of these T cells was observed in response to a range of p 2 peptide concentrations (data not shown). Thus, the biological activity displayed by the analogue peptide is not the result of a contaminant. Biotinylated peptides p 2, 28–40 and p 2, 28–40A34,36 were purchased from Advanced Biotechnology Centre (Imperial College School of Medicine at Charing Cross Hospital, London, U.K.).

Isolation of p 2, 28–40-reactive T cell clones

T cell clones AC1.1, AC34.11, AC34.25, and AC34.26 were isolated from a HDM-allergic individual (DR7, 11; DQ2, 7; DPB1*0101/0402) by limiting dilution cloning from a long-term Der p 2-specific T cell line, as described previously (27). The T cell clones (5–10 × 10^6) were maintained in complete medium: RPMI 1640 medium supplemented with 2 mM l-glutamine (Life Technologies, Paisley, U.K.) and 5% heat-inactivated human AB serum (Sigma Pharmaceuticals, Poole, U.K.) by weekly stimulation with peptide 28–40 at predetermined optimal concentrations (1–10 μM), irradiated (3000 rad), autologous PBMC (5 × 10^6/m) as APCs, in combination with IL-2 (Lymphocult-T; Biotest Ltd., Solihull, U.K.). In all experiments, T cells were restimulated for 7 days after the last antigen stimulation before use in the experiments. All clones are HLA class II restricted by HLA-DRB1*1101.

T cell anergy induction and proliferation assays

T cell anergy was induced by incubating T cells (1–2 × 10^6/ml) with supraimmunogenic concentrations (25–200 μM) of peptide in the absence of professional APCs for a minimum of 16 h or a maximum of 6 days. As a negative control, T cells were incubated in the absence of peptide or in the presence of an irrelevant peptide. After the incubation period, T cells were washed and assayed. Cellular proliferation was measured in a standard proliferation assay by culturing T cells (2 × 10^6/well) in round-bottom 96-well microtiter plates (Nunc, Roskilde, Denmark) with peptides presented by the irradiated (6000 rad) autologous EBV-transformed B cell line ACE, or with IL-2 in a total volume of 200 μl of complete medium. Background values were determined by incubating T cells with peptide 28–40 without optimal concentrations of peptide) or anergized (1 × 10^6 T cells/ml with 50 μM of peptide). IFN-γ, IL-4, IL-5, and IL-10 were measured by capture ELISA according to the PharMingen ELISA protocol using the following mAb sets and recombinant human cytokines (PharMingen): NIB42 and 4S.B3 (IFN-γ), 8D4-A, and MP4-25D2 (IL-4), TRFK5 and JES1-5A10 (IL-5), JES3-9D7 and JES3-6B11 (IL-10). TGF-β was assayed using the TGF-β-responsive cell line MV 1 Lu (NBL-7) as described previously (30).

Results

Proliferation, IFN-γ and IL-5 production by Der p 2-specific CD4+ T cells stimulated with native (p 2, 28–40) and analogue (p 2, 28–40A34,36) peptides

AC1.1, AC34.11, AC34.25, and AC34.26 are functionally representative of the human CD4+ T cell repertoire reactive with Der p 2 (27) and were isolated from a HDM-reactive allergic asthmatic individual. These T cells clones were selected for this study because they all recognize the same T cell epitope in Der p 2 (residues 28–40) and 101–129 (25–27). We have previously reported that two peptide analogues of p 2, 28–40 with single alanine substitutions at positions 34 and 36 enhanced IFN-γ but not IL-4 production by a clone of specific TH0 cells (28).

Down-regulation of the TCR/CD3 complex was determined as described previously (29). Briefly, autologous irradiated ACE (1 × 10^7/ml) were incubated in the absence or presence of increasing concentrations of native and analogue peptides (10^-3–10^-5 μg/ml), for 2 h. The cells were washed and mixed with T cells in a ratio of 1:2. After 24 h, cells were recovered and stained with α-CD3 (UCHT1; PharMingen, U.K.), CD3 fluorescence was analyzed on a FACSscan on gated live T cells using forward and 90-degree side scatter parameters. CD3 fluorescence of T cells incubated with unpulsed ACE represents the 100% value.

Peptide binding to HLA-DRB1*1101

Binding affinity of peptides p 2, 28–40 and its analogue p 2, 28–40A34,36 to HLA-DRB1*1101 was determined as follows: biotinylated versions of both peptides were incubated with live L-1101 cells (3 × 10^5 cells/well of a 96-well round-bottom tissue culture plate), over a range of concentrations, in a total volume of 200 μl RPMI 1640/1% FCS for 4 h. Cells were washed and incubated with 10 μg/ml avidin D FITC (Vector Laboratories, Peterborough, U.K.) for 30 min at 4°C and washed. Dead cells were excluded from the analysis by staining with 10 μg/ml propidium iodide. Background value was assessed by measuring fluorescence in the absence of peptide. To determine nonspecific peptide binding to L cells, untransfected L cells were incubated with or without 100 μM concentrations of each peptide and stained with 10 μg/ml avidin D FITC, as described above.

Measurement of IFN-γ, IL-4, IL-5, IL-10, and TGF-β in cell supernatants

Supernatants were collected at different time points after T cells were activated (1 × 10^6 T cells/ml with equal numbers of irradiated ACE, with or without optimal concentrations of peptide) or anergized (1 × 10^6 T cells/ml with 50 μM of peptide). IFN-γ, IL-4, IL-5, and IL-10 were measured by capture ELISA according to the PharMingen ELISA protocol using the following mAb sets and recombinant human cytokines (PharMingen): NIB42 and 4S.B3 (IFN-γ), 8D4-A, and MP4-25D2 (IL-4), TRFK5 and JES1-5A10 (IL-5), JES3-9D7 and JES3-6B11 (IL-10). TGF-β was assayed using the TGF-β-responsive cell line MV 1 Lu (NBL-7) as described previously (30).
Threshold effects of peptide stimulation on the modulation of Th0 cell effector function

To determine whether the modulation of T cell effector function was due to differences in Ag threshold, T cells were stimulated with increasing doses (10^{-2}–10^{-3} μM) of peptide. At equimolar concentrations, the magnitude of the proliferative response induced by either peptide was the same (Fig. 1A). Supernatants were collected from the T cell cultures and the levels of IL-5, IL-10, and IFN-γ were measured. The production of both IL-5 and IL-10 was similar in response to stimulation with native or analogue peptide at the same concentration (Fig. 1, B and C). Over the dose range 10^{-1}–10^{-2} μM, proliferation reached a plateau and then decreased, whereas IL-5 and IL-10 synthesis increased in parallel with peptide concentration. In contrast, IFN-γ production was enhanced by stimulation with p2,28–40A34,36 as compared with native peptide and was independent of Ag concentration in that the analogue induced a heteroclitic response at all of the doses investigated (Fig. 1D). Similarly, analysis of cell surface CD3 revealed that at equimolar concentrations the analogue peptide induced more marked down-regulation than the wild-type peptide (Fig. 1E).

### Table I. Effects of native p2, 28–40 and p2, 28–40A34,36 analogue on proliferation and cytokine production by human T cell clones representative of the Der p2 repertoire

<table>
<thead>
<tr>
<th>T Cell Clones</th>
<th>Parameters</th>
<th>Stimuli</th>
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<tbody>
<tr>
<td></td>
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<td>Medium</td>
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<tr>
<td>AC1.1</td>
<td>Proliferation (cpm)</td>
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<td></td>
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<td>IFN-γ (pg/ml)</td>
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<td></td>
<td>IL-5 (pg/ml)</td>
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<tr>
<td></td>
<td>IFN-γ (pg/ml)</td>
<td>0</td>
</tr>
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<td>AC34.25</td>
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</tr>
<tr>
<td></td>
<td>IFN-γ (pg/ml)</td>
<td>0</td>
</tr>
<tr>
<td>AC34.26</td>
<td>Proliferation (cpm)</td>
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<tr>
<td></td>
<td>IL-5 (pg/ml)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>IFN-γ (pg/ml)</td>
<td>0</td>
</tr>
</tbody>
</table>

* T cells (2 × 10^4/well of a 96-well tissue culture plate) were incubated with equal numbers of irradiated autologous ACE in the absence or presence of 10 μM peptides. Supernatants were collected after 48 h from triplicate wells, pooled, and stored. They were assessed for the presence of cytokines by ELISA. Proliferation as correlated with [3H]thymidine incorporation (expressed as mean cpm of triplicate cultures) was measured after 72 h, with [3H]thymidine present for the last 6 h. One representative experiment of three is shown. SEM was <20%.

**FIGURE 1.** Relationship between proliferation, cytokine production, and TCR down-regulation upon stimulation of T cells by both native and analogue peptides. To determine proliferation and cytokine production, cloned T cells (AC1.1; 2 × 10^4/well) were incubated with equal numbers of autologous irradiated ACE in the absence or presence of increasing concentrations of p2, 28–40 (●) or p2, 28–40A34,36 (○) in 96-well plates. Proliferation, determined after 72 h, including a 6-h [3H]thymidine pulse, is shown in A, whereas B–D show IL-5, IL-10, and IFN-γ production, respectively, after 48 h, as measured by ELISA. TCR/CD3 down-regulation (E) was measured by FACScan on T cells stained with α-CD3-FITC after 24 h of incubation with peptide-pulsed, autologous irradiated ACE. CD3 levels are expressed as a percentage of maximum CD3 levels on T cells incubated with unpulsed ACE cells. Data are representative of at least three independent experiments. Proliferation is expressed as mean cpm of triplicate cultures. Cytokine production is expressed as mean concentration of duplicate cultures. SEM was <20% in all cases.
These observations imply that p2, 28–40A34,36 may have a higher affinity for TCR or increase TCR occupancy and, furthermore, that selected T cell effector functions have different signaling thresholds of induction.

It was also possible that the altered biological activity of the agonist p2, 28–40A34,36 may arise as the result of increased binding to DRB1*1101 as compared with native peptide. Therefore, the affinity of binding of p2, 28–40A34,36 and native peptide to cell surface HLA class II was determined. At the concentrations tested, the native peptide bound with similar affinity to that of the analogue (Table II), suggesting that the modified effector functions induced by p2, 28–40A34,36 were not due to altered HLA class II binding affinity.

**Induction of anergy with native peptide and p2, 28–40A34,36.**

In the absence of APCs, T cells of AC1.1 were exposed to increasing concentrations of peptide and then restimulated with APCs, native peptide, analogue peptide, or with IL-2 alone (21). At every dose tested (25–200 μM), p2, 28–40A34,36 induced more profound anergy than the native peptide (Fig. 2). At the lowest peptide concentration tested (25 μM), stimulation with p2, 28–40A34,36 resulted in a reduction in Ag-dependent proliferation (>8-fold) compared with native peptide (1- to 2-fold) at the same dose. Even at 200 μM, the degree of anergy induced by native peptide was less than that mediated by p2, 28–40A34,36. Both peptides anergized the response to restimulation with p2, 28–40 as well as to p2, 28–40A34,36 (Fig. 2). Control responses to exogenous IL-2 were similar after exposure to either ligand.

**Cytokine production during the induction of anergy with native peptide and p2, 28–40A34,36.**

Inhibitory cytokines have been implicated in peripheral tolerance (31), therefore, we were prompted to investigate TGF-β and IL-10 secretion by Der p 2-specific Th0 cells exposed to concentrations of the native and agonist peptide that induce anergy. The principal aim of these experiments was to determine whether, as a consequence of anergy induction, the T cells adopt a regulatory phenotype characterized by the synthesis of TGF-β and IL-10 (31). Similar levels of TGF-β were induced by native peptide and p2, 28–40 A34,36 during both the induction of anergy and activation (Table III). Exposure to anergizing concentrations of either peptide stimulated the production of IL-10 that was markedly higher (>3-fold) than the levels that were detectable in activation and, furthermore, the analogue was the more potent (>22%) of the two peptides. Whether under conditions of anergy induction or activation, p2, 28–40A34,36 induced elevated IFN-γ secretion compared with the native peptide; however, in activation the comparable levels for each peptide were higher (Table III). Levels of IL-5 production during the induction phase of anergy in response to either peptide were the same and parallel previous observations on IL-4 secretion (32).

**Modulation of the cell surface phenotype during activation and the induction of anergy with native peptide and p2, 28–40A34,36.**

Membrane expression of the costimulatory receptor (CD28) and ligands (CD80 and CD86) was determined 24 h after activation or anergy induction with native peptide and p2, 28–40A34,36, with resting T cells serving as the background control (Fig. 3). Compared with resting T cells, the expression of both CD80 and CD86 was increased following activation with either peptide, and, for each of these cell surface proteins, levels were marginally higher in response to stimulation with p2, 28–40A34,36. During anergy induction, cell surface CD86 was increased, but expression was less than that present on activated T cells, whereas CD80 remained essentially at resting levels. A marginal increase in CD28 levels was detectable on activated as compared with resting and anergic T cells. No differences in expression of CD28, CD80, or CD86 on T cells rendered anergic by either peptide were observed. The peptide analogue, as compared with native peptide, induced more
marked down-regulation of CD3 on both activated and anergic T cells, which was more pronounced during the induction of anergy (Figs. 1E and 3). CD25 expression was increased during activation and anergy induction, with no marked differences between the peptides.

Discussion

The group 2 allergen, Der p 2, is a major target Ag of the human immune response to HDM, and within this protein an immunodominant T cell epitope (p 2, 28–40), defined at the population level, is located at residues 28–40 (25, 27). Previously, we have demonstrated that peptide analogues of p 2, 28–40, with single alanine substitutions at either position 34 or 36, increased IFN-γ production by human Th0 cells, but had no effect on IL-4 production (28). This finding prompted us to construct and investigate the immunological activity of a peptide analogue (p 2, 28–40A34,36), in which both residues at positions 34 and 36 have been substituted with alanine. In the present study, we report that this structural analogue, compared with the native peptide and the previously defined analogues of p 2, 28–40 (28), has profound regulatory effects on the qualitative nature of T cell responses in vitro.

The patterns of proliferation and cytokine production by a panel of human Th2/Th0 cell clones isolated from a HDM atopic individual were analyzed following stimulation with p 2, 28–40A34,36 in the presence of APCs. Although differences were observed between individual T cell clones, which may reflect variations in TCR structure, the overall effect was to promote a Th1 functional phenotype. At equimolar concentrations over a dose range of 5 logs, p 2, 28–40A34,36 induced enhanced IFN-γ production in comparison to native peptide, and the effect was also paralleled by increased down-regulation of membrane CD3. This result suggests a different threshold of signaling arising from changes in the affinity of interaction between TCR and its ligand (33), rather than merely from the amount of ligand available, as has been reported in other studies (34). In support of this interpretation is the finding that p 2, 28–40A34,36 and native peptide bind to DRB1*1101 with similar affinities. Since only very few peptide-MHC complexes are required to achieve full T cell activation, a minimal reduction in their numbers should not affect T cell activation or the induction of anergy (35). Furthermore, it has recently been reported that TCR/CD3 down-regulation as a result of peptide/MHC recognition is a direct measure of TCR occupancy (29). In that study, a direct correlation between the extent of TCR down-regulation and IFN-γ production was demonstrated, which is in agreement with our findings here. Furthermore, Valitutti and Lanzavecchia (36) reported that stimulation of the T cells with a partial agonist was 1000-fold less effective in TCR/CD3 down-modulation compared with the native peptide and this corresponded to substantially reduced IFN-γ secretion. Conversely, we find that amino acid substitutions in native T cell ligands need not necessarily lead to loss of selected T cell function but, instead, may result in gain of function due to increased TCR/MHC/peptide avidity. Ligands that display such properties are termed superagonists, and this is well illustrated by the recent observation that a set of altered TCR ligands derived from myelin proteolipid protein, in which the primary TCR contact residue had been replaced with a range of different amino acids, revealed a hierarchy in T cell proliferation and cytokine production (37). Furthermore, altered ligands with hyperstimulatory activity induced a shift in cytokine profiles toward the production of Th1 cytokines. However, the pattern we observed for IFN-γ secretion was not repeated for IL-5 or IL-10, and for both of these cytokines production increased with peptide concentration and exhibited the

![Image](http://www.jimmunol.org/Downloaded_from/http://www.jimmunol.org/)

**FIGURE 3.** Comparison of cell surface protein expression levels on T cells stimulated or anergized with native or analogue peptide. T cells (1 × 10^6/ml) were incubated in the absence or presence of peptides (p 2, 28–40, ■ p 2, 28–40A34,36, □ alone (50 µM) or peptide (5 µM) presented by irradiated ACE (1 × 10^9/ml) for 24 h. Cells were washed and incubated with mAbs for 30 min at 4°C. Dead cells were excluded with propidium iodide. Fluorescence intensity was determined on 5000 viable cells by flow cytometry and was measured on a linear scale. The background control of resting T cells cultured with ACE in the absence of Ag is shown (□). Results were reproduced in two additional experiments.
same sensitivity to both peptides, which implies that ligand density is influencing the pattern of response, and that the threshold of signaling is different than that of IFN-γ (19, 34).

While vaccination with altered TCR ligands to promote the production of Th1 cytokines may offer one approach to allergen immunotherapy, an alternative option is to induce anergy in specific Th2 cells. It has been reported that patients receiving hymenoptera venom immunotherapy exhibit suppressed Ag-specific T cell proliferation and cytokine production (12). This finding, along with the observation that exogenous IL-2 and IL-15 restored both proliferation and Th1 cytokine synthesis, suggested that the peripheral T cells had been anergized (12). We observed, in vitro, that the p 2, 28–40 analogue was a markedly more potent inducer of anergy than the native peptide, again reflecting increased affinity for TCR. From recent clinical studies on venom immunotherapy, there is evidence that IL-10 production by T cells, B cells, and monocytes is elevated (38). We also investigated IL-10 synthesis following exposure of the T cells to anergizing doses of p 2, 28–40 analogue and could detect levels that exceeded those induced by the native peptide. It has been noted that human CD4⁺ T cells cultured in IL-10 became unresponsive to Ag restimulation (29), while murine cells adopt a regulatory phenotype capable of suppressing Th1 immunity (39). Thus, enhanced autocrine secretion of IL-10 may account for the more profound level of anergy induced by the analogue. There is also evidence that dendritic cells cultured in IL-10 adopt a tolerogenic phenotype and that this occurs as the result of the down-regulation of costimulatory ligands (40). Analysis of the surface phenotype of the anergic T cells revealed a marked increase in CD86 expression compared with resting T cells, however, this did not differ between p 2, 28–40 analogue and native peptide. A recent study analyzing the function of CD86 expressed as a transgene on T cells revealed that anergy induction was not dysregulated (41).

Similar to IL-10, the cytokine TGF-β has been implicated in the delivery of inhibitory signals that mediate peripheral tolerance (42). However, for the allergen-specific T cells investigated here, there was no evidence of increased TGF-β synthesis after stimulation with either the analogue or native p 2, 28–40 peptide under conditions of activation or tolerance. This is in contrast to reports that cloned human Th0 cells, stimulated with a partial agonist peptide, secreted TGF-β selectively (43) and that TGF-β production is enhanced in anergic T cells (44). Thus, it seems unlikely that TGF-β contributes to the induction of anergy in our model or accounts for the increased tolerogenic activity of p 2, 28–40 analogue (43).

Targeting allergen-specific T cells in a manner which leads to either a decrease in the ratio of Th2:Th1 cytokines, or to the induction of T cell anergy, should have beneficial effects in allergic disease. Recent in vitro studies have confirmed that functionally polarized human T cells retain the capacity to modulate their cytokine pattern when stimulated with altered TCR ligands based on allergen peptides such as the group I allergen of the HDM (45), Japanese cedar pollen allergen (46), and phospholipase A₂ (34). In each of these, IFN-γ production was enhanced but appeared to occur as the consequence of altered MHC class II binding. The mode of action of the analogues differed in that they promoted IL-12 production by APCs (45) or, through reduced MHC class II binding, selectively inhibited Th2 cytokine synthesis by disabling TCR signaling (47). In support of our findings, it was recently shown that an analogue of the allergenic peptide OVA₂₃₂–₃₃₉, which was found to be a superagonist capable of promoting immunity in vitro, could inhibit allergic inflammation in a murine model of OVA-induced asthma (48). Moreover, this peptide analogue showed similar binding affinities to MHC class II as the native peptide. These studies on the modulation of specific effector function of allergen-specific T cells by altered TCR ligands, taken together with these reported here, imply that analogue peptides have a potential therapeutic application. However, at present, evidence that they can modulate or tolerate Th2 immunity in a clinical setting and thus lead to safe and effective immunotherapy for allergic patients is required.

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