

Reversal of human allergen-specific CRTH2⁺ T_H2 cells by IL-12 or the PS-DSP30 oligodeoxynucleotide

Francesco Annunziato, PhD,^a Lorenzo Cosmi, MD,^a Roberto Manetti, MD,^a

Francesca Brugnolo, MD,^a Paola Parronchi, MD,^a Enrico Maggi, MD,^a

Kinya Nagata, PhD,^b and Sergio Romagnani, MD^a Florence, Italy, and Saitama, Japan

Background: The chemoattractant receptor homologous molecule expressed on T_H2 cells (CRTH2) is a receptor for prostaglandin D₂, which among human T cells is selectively expressed by T_H2 and type 2 cytotoxic effectors.

Objective: Our purpose was to assess whether the cytokine production profile of T_H2 effectors could be reversed by exploiting their selective expression of CRTH2.

Methods: CRTH2⁺ T cells were purified from the blood of allergic subjects, stimulated with the specific allergen in the absence or presence of IL-12, and assessed by flow cytometry at the single-cell level for their ability to produce IL-4 and/or IFN- γ after antigen or polyclonal stimulation.

Results: Both IL-12 and the PS-DSP30 oligodeoxynucleotide enabled CRTH2⁺ allergen-stimulated T_H2 cells to produce IFN- γ . This change in the profile of cytokine production by T_H2 cells from allergic subjects was related to the upregulation of IL-12 receptor β 2 chain and was associated with the loss of CRTH2.

Conclusions: These data demonstrate that the cytokine production pattern of fully differentiated T_H2 effectors can be changed to a less polarized profile, thus providing the physiologic basis for new immunotherapeutic strategies in allergic disorders. (J Allergy Clin Immunol 2001;108:815-21.)

Key words: Allergy, T_H2, CRTH2, IL-12, IL-12 receptor, IL-4, ODN, CD28

Atopic diseases are the result of T_H2-dominated responses to single or multiple environmental allergens. T_H2 effector cells indeed produce cytokines, such as IL-4, IL-5, and IL-13, which are responsible for the joint involvement of IgE-producing B lymphocytes, mast cells, and eosinophils in allergic inflammation.^{1,2} T_H2 cells migrate from peripheral blood to, and accumulate in, sites of allergic inflammation through interaction with various cellular and humoral components, such as selectins, integrins, and locally produced chemoattractants.³⁻⁷ Some

Abbreviations used

APC: Antigen-presenting cell

CRTH2: Chemoattractant receptor of T_H2 cells

Der p 1: *Dermatophagoides pteronyssinus* group 1

IL-12R: IL-12 receptor

ODN: Oligodeoxynucleotide

Pen G: Penicillin G

TCR: T-cell receptor

UCB: Umbilical cord blood

chemokines, such as eotaxin, macrophage-derived chemokine/thymus-activated and regulated chemokine, and I-309, interact with their specific receptors (CCR3, CCR4, and CCR8, respectively), which are preferentially expressed on the surface of T_H2 cells.⁵⁻⁷

More recently, a novel chemoattractant receptor, CRTH2,⁸ whose ligand has been shown to be prostaglandin D₂,⁹ was found in T_H2 cells. Interestingly, CRTH2 is expressed not only in T_H2 cells but also in type 2 T cytotoxic cells, eosinophils, and basophils.^{8,10,11} More importantly, among CD4⁺ T cells, CRTH2 appears to be selectively expressed by T_H2 cells, whereas it is absent from both T_H1 and T_H0 cells.¹¹ Thus, CRTH2 might represent an important tool for a better understanding of the physiology of human T_H2 cells.

In this study, we have exploited the selective expression of CRTH2 by T_H2 cells to determine whether the cytokine profile of circulating T_H2 cells, including those specific for allergens, could be changed. The results demonstrate that both IL-12 and the PS-DSP30 oligodeoxynucleotide (ODN) were capable of inducing allergen-specific T_H2 cells to produce IFN- γ . The change in the cytokine profile was mediated by the expression on T_H2 cells of the IL-12 receptor (IL-12R) β 2 chain, which was induced by T-cell receptor (TCR) plus CD28 signaling and stabilized by IL-12. This shifting effect was also associated with the loss of CRTH2 by T_H2 cells. Thus, our data support the concept that even established T_H2 responses against allergens can be modified by appropriate therapeutic strategies.

METHODS

Subjects

Samples used in this study were obtained from peripheral blood of informed adult healthy donors, atopic donors sensitive to *Dermatophagoides pteronyssinus* group 1 (Der p 1) allergen, and 1

From ^athe Department of Internal Medicine, Section of Clinical Immunology, Allergy and Respiratory Diseases, University of Florence, Italy, and ^bR & D Center, BML, Inc, Kawagoe, Saitama, Japan.

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Reprint requests: Sergio Romagnani, MD, Dipartimento di Medicina Interna, Università di Firenze, Viale Morgagni, 85 Firenze-50134, Italy.

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penicillin-sensitive donor and from umbilical cord blood (UCB) of newborns in accordance with the ethical standards of the responsible regional committee on human experimentation.

Generation of T_H1- and T_H2-polarized T-cell lines from UCB lymphocytes

Polyclonal CD4⁺ T-cell lines were generated from UCB mononuclear cell suspensions from 12 newborns, as described previously.¹² Briefly, CD4⁺CD45RA⁺ T cells were purified by negative magnetic selection through use of the Magnet Activated Cell Separation (MACS) system (Miltenyi Biotec, Bergisch Gladbach, Germany), after a 2-step incubation with a mixture of anti-CD8, anti-CD14, anti-CD20, anti-CD56, and anti-CD45R0 mAbs (Becton-Dickinson, San Jose, Calif) and anti-glycophorin A and B mAbs (Signa Chemical, Milan, Italy) followed by goat antimouse IgG conjugated with magnetic beads (Miltenyi Biotec). Recovered cells (>99% CD3⁺CD4⁺CD45RA⁺) were then stimulated with PHA (Gibco Life Technology, Gaithersburg, Md; 0.1% v/v) and IL-2 (Eurocetus, Milan, Italy; 20 U/mL) in the absence or presence of IL-4 (R&D Systems, Minneapolis, Minn; 2 ng/mL) or IL-12 (R&D Systems; 2.5 ng/mL) or in the absence or presence of both cytokines. In 6 experiments, the CRTH2⁺ cells of IL-4-conditioned cultures were purified through use of the MACS system (see the specific paragraphs in the Methods section) and subjected to a second round of stimulation by PHA/IL-2 in the absence or presence of IL-4 or IL-12 in RPMI medium containing 10% heat-inactivated FCS.

Positive selection of CRTH2⁺ CD4⁺ T cells

Positive selection of CRTH2⁺ CD4⁺ T cells was performed with high-gradient magnetic cell sorting (through use of MACS), as described by Scheffold et al.¹³ Briefly, CD4⁺ T cells from IL-4-conditioned cultures or PBMC suspensions from atopic donors were incubated for 20 minutes with anti-CRTH2 mAb,¹¹ extensively washed, and then incubated for additional 20 minutes with goat antirat polyclonal antibody conjugated to colloidal superparamagnetic microbeads according to the MACS system (Miltenyi Biotec). After washing, cells were separated on an LS column and inserted into a MidiMACS magnet (Miltenyi Biotec). The purity of recovered cells was consistently higher than 98%.

Generation of short-term antigen-specific or polyclonal T-cell lines from purified circulating CRTH2⁺ T cells

PBMC suspensions were obtained from allergic donors by centrifugation on Ficoll-Hypaque gradient (Nycomed, PHARMA AS, Oslo, Norway). After being sorted for CRTH2 expression as described above, the cells were stimulated with the appropriate allergen (5 µg/mL of Der p 1 or 500 µg/mL of penicillin G [pen G]) and autologous irradiated (6000 rad) PBMCs as antigen-presenting cells (APCs) in the absence or presence of human recombinant IL-12 (2.5 ng/mL) or PS-DSP30 ODN (10 µg/mL), as detailed elsewhere.^{12,14} In some experiments, parallel cultures were done in the presence of anti-CD80 (5 µg/mL) and anti-CD86 (5 µg/mL) neutralizing or isotype control mAbs (BD PharMingen, Franklin Lakes, NJ). After 5 days of culture, activated T cells were expanded in the presence of recombinant IL-2 (20 U/mL). The cells were monitored every 2 days for IL-12Rβ2 chain and CRTH2 expression. On day 12, all T-cell lines were stimulated for detection of intracellular IL-4 and IFN-γ synthesis, as described below. The TCRVβ repertoire of T-cell blasts from 4 T-cell lines (3 derived from Der p 1-sensitive atopic donors and 1 from a pen G-allergic subject) was monitored by flow cytometry at the beginning and on day 12 of culture through use of a panel of FITC- or phycoerythrin-conjugated anti-TCRVβ antibodies (Serotec, Oxford, United Kingdom).

Polyclonal T-cell lines were obtained from purified CRTH2⁺ cells of 5 normal donors by their stimulation on plates coated with anti-CD3 (5 µg/mL) plus anti-CD28 (10 µg/mL) or isotype control mAbs in the absence or presence of IL-12 (2.5 ng/mL). The cells were monitored every 3 days for IL-12Rβ2 chain expression and cytokine production profile.

Flow cytometric analysis

Analysis of cell surface molecules and intracellular cytokines was performed by means of a BD-LSR cytofluorometer (BD PharMingen), as described previously.¹² Polyclonal cell stimulation for cytokines induction was performed as described previously.¹⁴ Briefly, 1 × 10⁶ cells were stimulated with phorbol 12-myristate 13-acetate (PMA; 10 ng/mL) plus ionomycin (1 µmol/L) for 4 hours; during the final 2 of these hours, this was in the presence of brefeldin A (5 µg/mL). For the intracytofluorometric analysis of IFN-γ and IL-4 synthesis in antigen-specific T-cell lines, 1 × 10⁶ cells were stimulated with Der p 1 (5 µg/mL) or pen G (0.5 mg/mL) and autologous APCs (1:1 ratio) for 8 hours; during the final 6 of these hours, this was in the presence of brefeldin A (5 µg/mL).

Statistical analysis

Statistical analysis was performed through use of the Student *t* test for paired data.

RESULTS

CRTH2 expression by activated naive T helper cells is upregulated by IL-4 and inhibited by IL-12

We first investigated the mechanisms that regulate CRTH2 expression in primed human naive T helper cells. To this end, UCB CD4⁺ T cells from 12 different donors were stimulated with PHA and IL-2 in the absence or presence of IL-4, IL-12, or IL-4 plus IL-12, and CRTH2 surface expression was assessed at different time intervals. As shown in Fig 1, A, CRTH2 was expressed only by CD4⁺ T cells from IL-4-conditioned cultures, whereas CD4⁺ T cells stimulated in the absence of IL-4 or in the presence of both IL-4 and IL-12 showed no CRTH2 expression. CRTH2 expression started between day 3 and day 4 of culture and plateaued until day 12. These data indicate that CRTH2 expression is dependent on the presence of IL-4 and that the contemporaneous presence of IL-12 at the time of stimulation counteracts the ability of IL-4 to induce CRTH2 expression.

To establish whether IL-12 was able to downregulate CRTH2 expression even if added later after the initial conditioning with IL-4, CRTH2-expressing cells present on day 3 in IL-4-conditioned cultures were positively sorted; they were then incubated with medium alone, with IL-4, or with IL-12 and assessed for CRTH2 expression on days 6 and 9. As shown in Fig 1, B, the addition of IL-12 significantly reduced the number of CRTH2-expressing cells on day 9 in comparison with parallel cultures supplemented with either IL-4 or medium alone. The effect of IL-12 was not due to the inhibition of IL-4 production by CRTH2-expressing cells, because the numbers of these cells were even greater on day 9 than on day 3 or day 6 (Fig 1, C); instead, the effect appeared to be related to the induction in the same cells of the ability to produce IFN-γ (Fig 1, D).

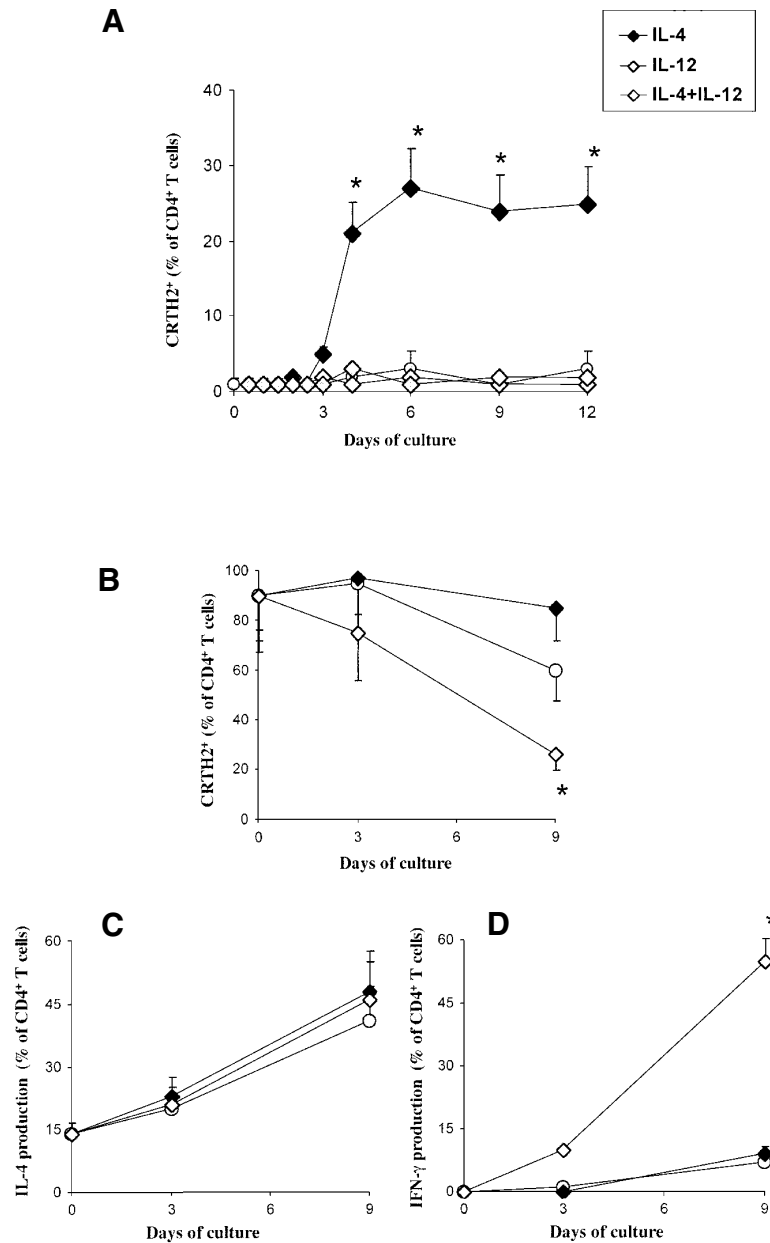


FIG 1. Regulatory effects of IL-4 and IL-12 on CRTH2 expression by activated naive CD4⁺ T cells. **A**, Purified CD4⁺CD45RA⁺ UCB T cells were stimulated with PHA and IL-2 in medium alone, IL-4, IL-12, or IL-4 plus IL-12 and assessed at the indicated days for surface CRTH2 expression by flow cytometry. Results represent mean values ($\pm$ SDs) of CRTH2⁺ cells obtained in 12 separate experiments. **B**, CD4⁺CD45RA⁺ UCB T cells were stimulated with PHA and IL-2 in the presence of IL-4. After 3 days, CRTH2⁺ cells were positively sorted and incubated again with medium alone, IL-4, or IL-12 and assessed at the indicated days for CRTH2 expression. **C**, Intracytofluorometric analysis of the production of IL-4 in response to stimulation with PMA plus ionomycin by the same cells. **D**, Analysis of the production of IFN- γ . The results represent mean values ($\pm$ SDs) obtained in 6 separate experiments. * $P < .01$ in comparison with values found in cultures containing medium alone.

CRTH2-expressing allergen-specific T_H2 cells are induced by IL-12 to produce IFN- γ

We then tried to answer the question of whether even allergen-specific T_H2 effectors from atopic subjects could be induced to produce IFN- γ . To this end, circulating

CRTH2⁺ cells from 3 Der p 1-sensitive subjects were positively sorted and stimulated with Der p 1 plus irradiated autologous PBMNC as APCs in the absence or presence of IL-12 and, for 2 of them, in the presence of PS-DSP30, an ODN that has previously been shown to induce the production of IL-12 by APCs and to shift the in vitro differen-

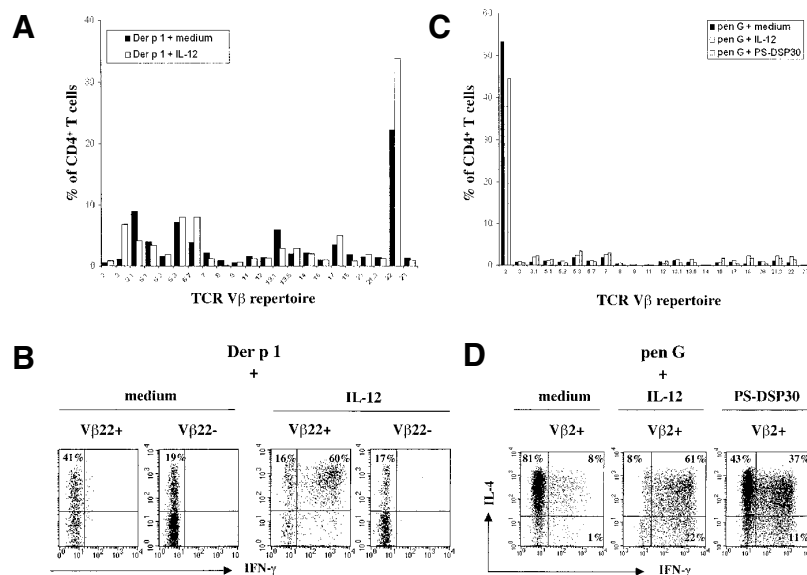


FIG 2. Induction of IFN- γ production in purified CRTH2 $^{+}$ CD4 $^{+}$ T cells from allergic subjects by allergen stimulation in the presence of IL-12 or PS-DSP30 ODN. CRTH2 $^{+}$ cells positively sorted from PBMCs of 1 Der p 1-sensitive subject and 1 penicillin-sensitive subject were stimulated with Der p 1 or pen G in the absence or presence of IL-12 (the latter also with the PS-DSP30 ODN). **A**, TCRV β repertoire of Der p 1-stimulated CD4 $^{+}$ T cells after 12 days of culture. **B**, IL-4 and IFN- γ intracellular synthesis in response to 8 hours of Der p 1-specific stimulation (see Methods section). Cell were analyzed gating on V β 22 $^{+}$ and V β 22 $^{-}$ T-cell blasts. **C**, TCRV β repertoire of pen G-stimulated CD4 $^{+}$ T cells after 12 days of culture. **D**, IL-4 and IFN- γ production in response to 4 hours of stimulation with PMA plus ionomycin of pen G-specific T cells. Cells were analyzed gating on V β 2 $^{+}$ T-cell blasts.

tiation of allergen-specific T cells from the T $_{H2}$ to the T $_{H1}$ profile.¹⁴ On day 12, Der p 1-stimulated CRTH2 $^{+}$ CD4 $^{+}$ T cells were assessed for their TCRV β repertoire, CRTH2 expression, and profile of cytokine production. In all cases, a remarkable increase (>20 times) in the number of cells showing a given TCRV β (V β 22, V β 3.1, and V β 6.7) in comparison with the starting population was observed (Table I and Fig 2, A), suggesting that allergen stimulation had indeed expanded the Der p 1-specific T-cell population. To support this finding, all cultures were then assessed for the ability to produce IL-4 and IFN- γ after 8 hours of stimulation with Der p 1 under MHC-restricted conditions of both Der p 1-specific and -nonspecific populations. Virtually all Der p 1-specific T cells from cultures performed in the absence of IL-12 or the PS-DSP30 ODN produced IL-4 but not IFN- γ . By contrast, a high proportion of Der p 1-specific T cells from cultures conditioned with IL-12 or the PS-DSP30 ODN showed the ability to produce IFN- γ in addition to IL-4 (Table I and Fig 2, B). Similar results were obtained in parallel cultures performed through use of purified and not irradiated autologous CD14 $^{+}$ cells instead of autologous irradiated PBMCs as APCs (data not shown).

To exclude the possibility that the effect induced by IL-12 or the PS-DSP30 ODN was a unique property of Der p 1-specific T cells, the same type of experiment was conducted with purified CRTH2 $^{+}$ cells from a penicillin-allergic subject. To this end, positively sorted CRTH2 $^{+}$ CD4 $^{+}$ circulating T cells from this subject were cultured for 12 days with pen G under MHC-restricted conditions in the absence or presence of IL-12 or the PS-DSP30

ODN. On day 12, a remarkable enrichment for V β 2 $^{+}$ CD4 $^{+}$ T cells was observed in unconditioned cultures as well as in IL-12- or PS-DSP30 ODN-conditioned cultures (Fig 2, C). When V β 2 $^{+}$ cells were examined for their ability to produce IL-4 and IFN- γ , virtually all CD4 $^{+}$ T cells from unconditioned cultures produced IL-4 but no IFN- γ , whereas most CD4 $^{+}$ T cells from cultures conditioned with either IL-12 or PS-DSP30 ODN produced both IL-4 and IFN- γ or even IFN- γ alone (Fig 2, D).

IL-12-stimulated IFN- γ production by T $_{H2}$ cells is due to IL-12R β 2 chain expression and is associated with the loss of CRTH2

The mechanisms responsible for the IL-12-mediated induction of IFN- γ production in allergen-specific T $_{H2}$ cells were then investigated. To this end, we examined the expression of the IL-12R β 2 chain by circulating CRTH2 $^{+}$ CD4 $^{+}$ T cells from 4 Der p 1-sensitive subjects before and after their stimulation with Der p 1 under MHC-restricted conditions in the absence or presence of IL-12. As shown in Fig 3, A, the IL-12R β 2 chain was apparently absent in unstimulated CRTH2 $^{+}$ CD4 $^{+}$ T cells, but it became detectable in a substantial number of these cells on day 6 after stimulation with Der p 1 in the absence, and even more so in the presence, of IL-12. However, on the subsequent days of culture, the IL-12R β 2 chain expression was lost by CD4 $^{+}$ T cells from unconditioned cultures, whereas it was maintained by large numbers of cells in cultures conditioned with IL-12 (Fig 3, A). Of note, the IL-12R β 2 chain expres-

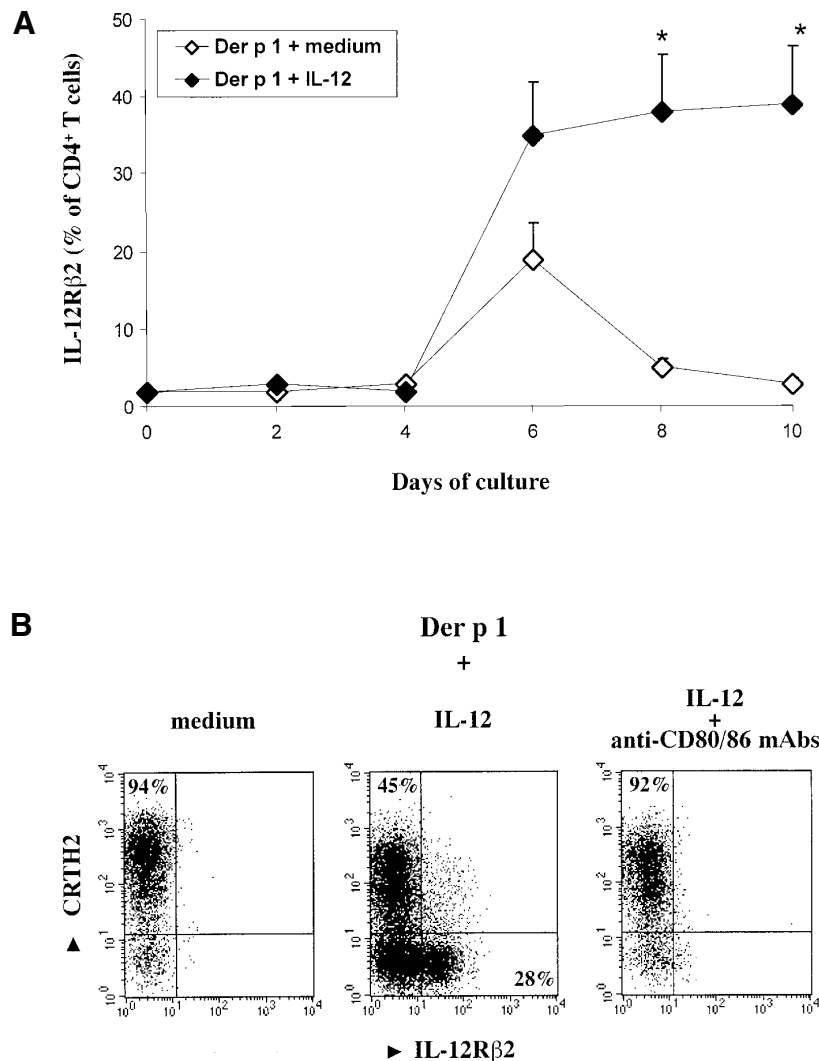


FIG 3. IL-12R β 2 chain expression by purified CRTH2⁺ T cells from Der p 1-sensitive subjects stimulated with Der p 1 in the absence or presence of IL-12. CRTH2⁺ T cells positively sorted from PBMCs of Der p 1-sensitive subjects were stimulated with Der p 1 in the absence or presence of IL-12. **A**, Assessment at different time intervals of surface IL-12R β 2 chain expression by flow cytometry. The results represent the mean values ($\pm$ SDs) obtained in 4 separate experiments. **B**, Effect of a mixture of anti-CD80 and anti-CD86 mAbs on the expression of CRTH2 and IL-12R β 2 chain in cultures of purified CRTH2⁺ cells stimulated for 10 days with Der p 1 in the presence of IL-12. * P < .01 in comparison with values found in cultures containing medium alone.

sion was associated with the loss of CRTH2 expression by CD4⁺ T cells from IL-12-conditioned cultures, but both IL-12R β 2 upregulation and CRTH2 downregulation were prevented by a mixture of anti-CD80 and anti-CD86 neutralizing antibodies (Fig 3, B).

We therefore hypothesized that TCR plus CD28 signaling induced on T_H2 cells by allergen stimulation resulted in the upregulation of IL-12R β 2 chain and that this made these cells susceptible to the activity of IL-12, which in turn was responsible for the induction of IFN- γ production. To investigate this possibility, CRTH2⁺CD4⁺ T cells from 5 normal subjects were positively sorted and stimulated with a mixture of insolubilized anti-CD3 and anti-CD28 antibodies in the absence or presence of IL-12 and then assessed at different time intervals for the

expression of IL-12R β 2 chain and their ability to produce IFN- γ . As shown in Fig 4, A, polyclonal stimulation resulted in a progressive increase in the number of CD4⁺ T cells expressing the IL-12R β 2 chain in both unconditioned and IL12-conditioned cultures. More importantly, the great majority of CRTH2⁺CD4⁺ T cells stimulated in the presence of IL-12 acquired the ability to produce IFN- γ in addition to IL-4 or to produce even IFN- γ alone (Fig 4, B). Of note, if the cells were removed from the wells containing the insolubilized anti-CD3 and anti-CD28 mAbs and cultured in wells containing only medium and IL-2, only those conditioned with IL-12 maintained the expression of the IL-12R β 2 chain on their surfaces, supporting the concept that IL-12 is also critical for the stabilization of its receptor (data not shown).

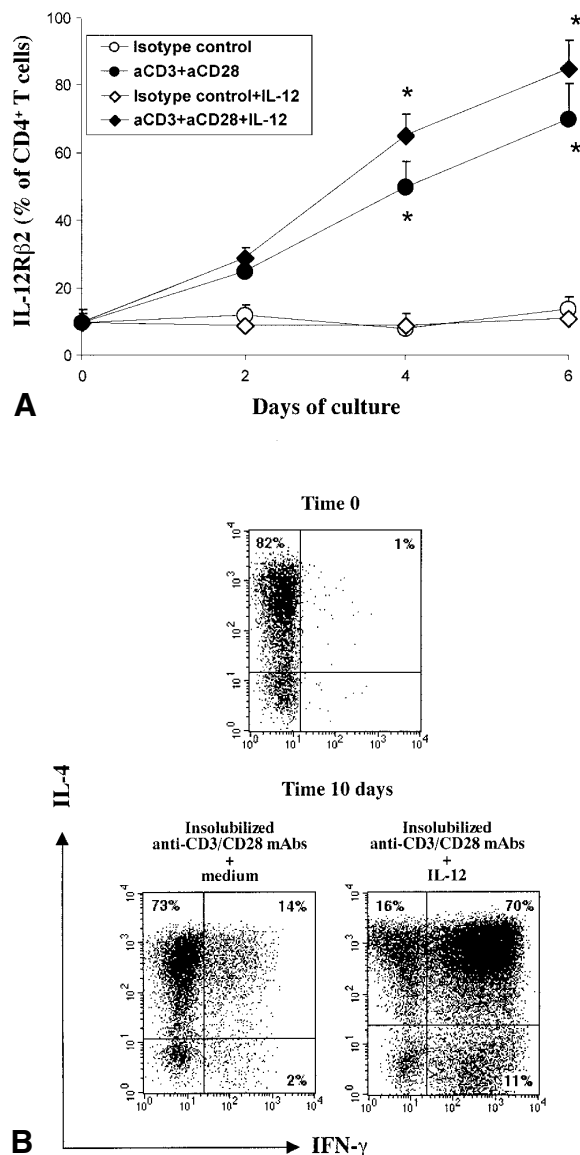


FIG 4. Induction of IL-12R β 2 chain expression in CRTH2⁺ T cells stimulated with anti-CD3 plus anti-CD28 mAbs. Purified CRTH2⁺ T cells were stimulated with a mixture of plate-coated anti-CD3 and anti-CD28 or isotype control mAbs. **A**, IL-12R β 2 chain expression as assessed at the indicated times by flow cytometry. Results represent the mean values ($\pm$ SDs) obtained in 5 separate experiments. **B**, IL-4 and IFN- γ production by the same cultures as evaluated by intracytofluorimetric analysis before and after 10 days of culture. One representative experiment is shown. * $P < .01$ in comparison with values found in isotype control cultures.

DISCUSSION

In a previous study, we demonstrated that CRTH2, which mediates the chemotactic activity of prostaglandin D₂,⁹ was selectively expressed by CD4⁺ and CD8⁺ T cells able to produce IL-4 and IL-13 but not IFN- γ , whereas it was absent from T_H0, T_H1, type 0 T cytotoxic, and type 1 T cytotoxic cells, thus supporting the concept that this molecule really represents a selective marker for human T_H2 and type 2 T cytotoxic cells.¹¹

TABLE I. Change in the cytokine profile of Der p 1-stimulated T cells induced by IL-12 or the PS-DSP30 ODN

Experiment no.	TCRV β *	Ratio between IL-4-producing and IFN- γ -producing T cells stimulated with Der p 1 in the presence of . . . †		
		Medium	IL-12	PS-DSP30 ODN
1	22	38.2	1.2	N.D.‡
2	3.1	35.1	1.0	2.0
3	6.7	43.9	1.1	1.9

CRTH2⁺CD4⁺ T cells were purified from the peripheral blood of 3 Der p 1-sensitive donors and cultured with Der p 1 in the absence or presence of IL-12 or the PS-DSP30 ODN. On day 12, T-cell blasts were assessed by flow cytometry for TCRV β repertoire and intracellular cytokine synthesis.

*The TCRV β that resulted, which was ≥ 20 times higher than that in the starting population (as expression of the Der p 1-induced T-cell expansion), is shown.

†The ratio between the number of CD4⁺ T cells showing intracellular IL-4 synthesis and the number of CD4⁺ T cells showing IFN- γ synthesis in response to 8 hours of stimulation with Der p 1 plus autologous APCs is shown.

‡Not done.

In the present study, we first investigated the mechanisms that regulate the expression of CRTH2 and then exploited this unique property of CRTH2 to investigate whether the cytokine secretion profile of allergen-specific T_H2 cells, as well as that of other T_H2 effectors, could be modified. The results showed that CRTH2 expression by naive T cells was dependent on IL-4 and was completely inhibited by IL-12. IL-12 inhibited CRTH2 expression by naive CD4⁺ T cells even if added in culture after they had been conditioned with IL-4, and it was also able to induce the loss of CRTH2 expression by established T_H2 cells stimulated with the specific allergen. Of note, CRTH2⁺CD4⁺ T cells stimulated with the specific allergen in the presence of IL-12 acquired the ability to produce IFN- γ in addition to IL-4, thus shifting from the T_H2 to the T_H0 profile of cytokine production. This finding is apparently at variance with the results of a previous study showing a lack of IL-12 signaling in human allergen-specific T_H2 cells.¹⁵ However, IL-12 was found to be able to induce transient IFN- γ production by established T_H2 clones,¹⁶ and in vitro IL-4-induced T_H2 cells could be reverted to T_H0 and T_H1 cells after a single IL-12 stimulation¹⁷ or after engagement of signaling lymphocyte activation molecule.¹⁸ Moreover, during murine leishmaniasis, IL-12 production resulted in the emergence of IFN- γ -producing cells and in the downregulation of T_H2 cell responses.^{19,20} More recently, IL-12-induced reversal of human T_H2 cells was found to be accompanied by full restoration of IL-12 responsiveness and loss of GATA-3 expression.²⁰ The results here reported clearly and definitively demonstrate that pure effector human T_H2 cells can be induced, at least in vitro, to produce IFN- γ when they are stimulated with the specific antigen in the presence of IL-12.

The results of this study also provide information on the mechanisms responsible for the shifting of T_H2 cells to the T_H0 cytokine profile. When CRTH2⁺CD4⁺ T cells from atop donors were stimulated with the specific allergen,

they acquired the ability to express the IL-12R β 2 chain, thus becoming susceptible to the activity of IL-12. The induction of a fully functional IL-12R appeared to be mediated by TCR plus CD28 signaling. First, among all CRTH2⁺ circulating T_H2 cells, only those specific for the allergen used for their stimulation acquired the ability to produce IFN- γ in the presence of IL-12. Second, the addition to cell cultures of a mixture of neutralizing anti-CD80 and anti-CD86 antibodies inhibited the upregulation of IL-12R β 2 chain in allergen-stimulated T_H2 cells. This finding is in keeping with recent observations showing the positive regulation of IL-12R expression by CD28/CD152 costimulation²¹ as well as the dependency of IL-12R β 2 chain upregulation in response to both TCR and CD28 signaling.^{22,23} Thus it can be suggested that stimulation with the specific allergen of circulating CRTH2⁺ allergen-specific T_H2 cells upregulates the expression of IL-12R, thus enabling these cells to respond to IL-12. If present in the microenvironment, IL-12 not only contributes to stabilization of the β 2 chain expression of its own receptor on T_H2 cells; it also induces the production of IFN- γ by T_H2 cells, thus promoting their shifting to a T_H0 pattern of cytokine production. Whether the stabilization of the IL-12R β 2 chain on the surface of T_H2 cells is a direct effect of IL-12 itself or is instead mediated by the activity of IFN- γ produced in response to IL-12 remains to be established. Of note, the acquisition of a functional IL-12R by T_H2 cells associated with their loss of CRTH2 expression provides additional support for the concept that CRTH2 is a highly selective marker of cells capable of producing IL-4 (and IL-13) but not IFN- γ —ie, pure T_H2 cells.

The results of this study appear to be of interest in view of the development of novel specific immunotherapeutic strategies designed to modify the pattern of cytokine production of allergen-specific T cells from allergic subjects. They demonstrate indeed that the same shifting effect induced by IL-12 on CRTH2⁺ allergen-specific CD4⁺ T cells from allergic subjects can be induced by their stimulation with the specific allergen in the presence of an appropriate ODN, PS-DSP30, which was previously shown to induce the production of IL-12 by APCs and to shift the in vitro differentiation of allergen-specific CD4⁺ T cells from the T_H2 to the T_H1 profile.¹⁴ Thus our data provide strong support for the concept that the utilization of allergen(s) mixed with, or conjugated with, appropriate adjuvants capable of inducing IL-12 production in the site of allergen presentation might be effective in favoring the shift of already established memory T_H2 cells, which are responsible for triggering the allergic inflammatory reactions in a less polarized and therefore potentially less dangerous effector response.

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