

Comparison of allergen-stimulated dendritic cells from atopic and nonatopic donors dissecting their effect on autologous naive and memory T helper cells of such donors

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Background: Because of their production of IL-12, mature dendritic cells (DC) are potent inducers of T_H1 responses. However, recent reports have demonstrated that DCs can also induce T_H2 differentiation.

Objective: In the current study we investigated which immune response is induced by DCs in naive CD45RA⁺ or memory CD45RO⁺ CD4⁺ T cells from atopic individuals (patients with grass pollen, birch pollen, or house dust mite allergy) compared with nonatopic control subjects.

Methods: Immature DCs, generated from peripheral blood monocytes from atopic and nonatopic donors, were pulsed with the respective allergen and fully matured. Then the mature DCs were cocultured in vitro with autologous naive (CD45RA⁺) and memory (CD45RO⁺) CD4⁺ T cells and cytokine and IgE production were measured by ELISA.

Results: After the second restimulation with allergen-pulsed DCs, naive as well as memory autologous CD4⁺ T cells from atopic but not from nonatopic donors showed an enhanced production of the T_H2-type cytokines IL-4, IL-5, and IL-10, resulting in an increased IgE production, whereas IFN- γ production and proliferation were not different. IL-12 production and surface marker expression of DCs derived from atopic and nonatopic donors did not differ and addition of neutralizing anti-IL-12 mAbs did not increase IL-4 but diminished IFN- γ production.

Conclusion: These data indicate that mature DCs are able to induce naive and activate allergen-specific T helper cells to produce T_H2 cytokines if the T cells are derived from atopic donors. This phenomenon is not due to diminished IL-12 production by DCs of atopic donors. (*J Allergy Clin Immunol* 2000;105:988-96.)

Key words: Dendritic cells, T_H2 cytokines, atopy

Dendritic cells (DC) are the most potent antigen-presenting cells in priming naive T and B lymphocytes and they play a key role in determining the type of an

Abbreviations used

DC: Dendritic cell
[³H]TdR: Tritiated thymidine
MIP-1 α : Macrophage inflammatory protein-1 α
RAST: Radioallergosorbent test

immune response.¹⁻⁴ Located in an immature state at the sites of antigen entry, such as Langerhans cells in the skin, they are specialized in antigen capture and processing. On stimulation by CD40 ligand (CD40L) or endotoxin they differentiate into mature DCs and migrate to the T-cell areas of lymphoid organs where they present processed peptides to naive T cells and initiate an immune response.⁵⁻⁷ Depending on the surrounding cytokine milieu secreted by neighboring cells (eg, macrophages, natural killer cells, and DCs), newly activated T cells commit into T_H1 or T_H2 cells that differ by the cytokine pattern they produce. T_H1-type cytokines (IL-2, IFN- γ) are responsible for cell-mediated immune responses, whereas T_H2-type cytokines (IL-4, IL-5, IL-10, IL-13) are associated with strong antibody responses including IgE-dependent allergies of the immediate type.⁸⁻¹⁰ Several studies have shown that the early presence of IL-4 induces T_H2 differentiation, whereas IL-12 promotes T_H1 development.^{11,12}

Activation of in vitro-generated DCs from precursors in peripheral blood or bone marrow by proinflammatory cytokines, CD40L, or LPS induces production of large amounts of IL-12, indicating their potent role in the induction of T_H1 cells.¹³⁻¹⁷ Besides their induction of type 1 cytokines, there are some reports demonstrating that DCs can also promote type 2 immune responses and that DCs are required for the development of allergic inflammation in response to inhaled allergen.^{18,19} Recently it has been suggested that distinct DC lineages, called DC1 and DC2, control the class of immune response in vivo and in vitro and that the developing T helper cells themselves regulate the survival of the respective DC subset.^{20,21} However, it is not clear whether these different types of DCs originate from the same precursor and whether the microenvironment influences their development. In this respect, it was demonstrated by Stumbles et al²² in the murine system that rest-

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ing respiratory tract DC preferentially stimulate T_H2 responses, which may be an inherent property, and that mobilization of T_H1 immunity requires additional microenvironmental stimuli.

In the current study we investigated whether in vitro-generated human monocyte-derived DCs induce different immune responses in naive and memory T helper cells from atopic patients with allergic rhinoconjunctivitis or asthma to grass pollen, birch pollen, rye pollen, or house dust mite compared with healthy nonatopic donors.

METHODS

Subjects and cell lines

Twenty nonatopic control subjects and 24 atopic donors with allergic rhinoconjunctivitis or asthma to grass pollen, birch pollen, rye pollen, or house dust mite were included in the study. Specific sensitization was documented by positive skin prick test to the respective allergen and detection of allergen-specific IgE in the sera (radioallergosorbent test [RAST] class ≥ 2). Allergens to which no preexisting allergic immune response is detectable (no positive skin test reactions and no allergen-specific IgE in the sera) were called "irrelevant" allergens. Therefore this does not imply that there was no exposure of the donor to this allergen.

Mouse fibroblastic L cells stably transfected with human CD40L or human CD32/Fc γ RII as control were kindly provided by Dr Pierre Garrone, Schering-Plough, Dardilly, France. L cells were cultured in RPMI 1640 medium (Gibco BRL, Life Technologies, Paisley, UK) supplemented with 10% FCS (Sigma, Deisenhofen, Germany) and expanded when confluent with Versene 1:5000 (Gibco BRL).

Generation of monocyte-derived DCs

PBMCs were isolated from heparinized blood by Ficoll-Paque 1.077 (Biochrom, Berlin, Germany) density centrifugation and CD14 $^{+}$ monocytes were enriched by adherence. Briefly, 1×10^7 PBMCs per well were incubated for 45 minutes in a 6-well plate (Costar, Bodenheim, Germany) in RPMI 1640 medium supplemented with 3% autologous serum at 37°C. After the nonadherent cells were washed with prewarmed PBS, the remaining monocytes (purity >90%) were incubated in 3 mL per well X-VIVO 15 (Bio-Whittaker, Walkersville, Md) supplemented with 1% heat-inactivated autologous serum, 1000 U/mL IL-4 (Strathmann Biotech, Hannover, Germany), and 800 U/mL GM-CSF (Leucomax, Sandoz, Nürnberg, Germany). Cells were fed with fresh medium every other day. After 7 days of culture the resulting immature DCs were pulsed with 10 μ g/mL grass pollen, birch pollen, rye pollen, or house dust mite (*Dermatophagoides pteronyssinus*) allergen extracts (ALK-Scherax, Hamburg, Germany) or 1 μ g/mL tetanus toxoid (Behring-Werke, Marburg, Germany) as control antigen. At the same time point DCs were additionally stimulated with 1000 U/mL TNF- α , 2000 U/mL IL-1 β (Strathmann Biotech), and 1 μ g/mL PGE $_2$ (Minprostin E $_2$, Pharmacia & Upjohn, Erlangen, Germany) to induce their full maturation. For some experiments 2 μ g/mL anti-IL-12 neutralizing mAb (R&D Systems, Wiesbaden, Germany) were added to the culture on day 7. Mature DCs were harvested 48 hours after stimulation (the yield of DCs out of 1×10^7 PBMCs was 0.5 to 1×10^6 DCs), washed twice and used for proliferation, cytokine, and IgE production assays.

T-cell and B-cell purification

CD45RA $^{+}$ and CD45RO $^{+}$ CD4 $^{+}$ T cells as well as CD19 $^{+}$ B cells were purified from PBMCs of the same donor with use of antibody-coated paramagnetic MicroBeads (MACS, Miltenyi Biotec, Ber-

gisch Gladbach, Germany) according to the protocol of the manufacturer. Separation was controlled by flow cytometry (purity of >98% CD4 $^{+}$ T cells, >95% CD45RA $^{+}$ CD4 $^{+}$ T cells, >95% CD45RO $^{+}$ CD4 $^{+}$ T cells, and >85% CD19 $^{+}$ B cells).

Proliferation assays

A total of 1×10^5 CD45RA $^{+}$ or CD45RO $^{+}$ CD4 $^{+}$ T cells were cocultured in 96-well plates (Costar) in triplicate with titrated numbers of autologous allergen-pulsed DCs in 200 μ L of X-VIVO 15. After 4 days the cells were pulsed with 1 μ Ci per well of tritiated thymidine ([^{3} H]TdR) ([methyl- ^{3} H]thymidine, ICN, Irvine, Calif) for 6 hours, harvested with a Skatron AS cell harvester (Skatron, Norway), and [^{3} H]TdR incorporation was evaluated in a β -counter (1205 Betaplate, LKB Wallac, Turku, Finland) after addition of scintillation liquid (Roth, Karlsruhe, Germany).

Cytokine production assays

A total of 5×10^5 CD45RA $^{+}$ or CD45RO $^{+}$ CD4 $^{+}$ T cells were cocultured in 48-well plates with 5×10^4 autologous allergen-pulsed DCs, pretreated with anti-IL-12, or untreated, in 1 mL of X-VIVO 15. T cells were weekly restimulated with newly generated allergen-pulsed DC and supernatants were collected 24 hours after the second and third stimulation. In some experiments 2 μ g/mL anti-IL-12 neutralizing mAb (R&D Systems) was added to the cultures.

IgE production assay

For determination of IgE production, 5×10^5 CD45RA $^{+}$ or CD45RO $^{+}$ CD4 $^{+}$ T cells were cocultured in 48-well plates with 1×10^5 CD19 $^{+}$ B cells and 5×10^4 autologous allergen-pulsed DCs in 1 mL of X-VIVO 15 in the presence of 1000 U/mL IL-4 (Strathmann Biotech) and 2.5×10^4 irradiated (7000 rad) CD40L L cells or CD32 L cells used as control, and supernatants were harvested after 12 days of culture.

Analysis of surface marker expression by flow cytometry

Surface phenotyping of DCs was performed by staining 5×10^4 cells with specific mouse antihuman mAbs for 20 minutes on ice. Antibodies used were CD80 (MAB104, Immunotech, Hamburg, Germany), CD83 (HB15a, Immunotech), CD86 (BU63, Camon, Wiesbaden, Germany), and rat antihuman HLA-DR (YE2/36HLK, Camon). After being washed with PBS containing 2% FCS, the cells were stained with phycoerythrin-conjugated donkey antimouse IgG (Dianova, Hamburg, Germany) and fluorescein (DTAF)-conjugated goat antirat IgG (Dianova). After 20 minutes the cells were washed and analyzed in a FACSCalibur (Becton-Dickinson, Mountain View, Calif) equipped with CELLQuest Software.

Quantification of cytokine and Ig production by ELISA

Human IL-12 p70 was measured by an ELISA Kit (Diacclone Research, Besançon, France) according to the manufacturers' descriptions (detection limit <3 pg/mL). Human IL-4, IL-5, IL-10, IL-12 p40, IFN- γ (PharMingen, San Diego, Calif), IL-6, IL-8, macrophage inflammatory protein-1 α (MIP-1 α) (R&D Systems), and IgE (Dako, Glostrup, Denmark) were measured by ELISA according to the instructions of the distributors of the pairs of antibodies used. Briefly, Maxisorp Nunc-Immunoplates (Nunc, Wiesbaden, Germany) were coated overnight with 2 μ g/mL (8 μ g/mL for IL-12p40) cytokine capture antibody diluted in 0.1 mol/L sodium phosphate, pH 9.0, at 4°C or with 10 μ g/mL antihuman IgE antibody diluted in PBS. The plates were blocked with PBS containing 1% BSA (Sigma) for 2 hours at 37°C. Serial dilutions of the standards (PharMingen, R&D Systems,

and DDV Biochemie, Marburg, Germany [human IgE]) and cell culture supernatants were incubated overnight at 4°C. The next day the plates were incubated at 37°C for 1 hour with 1 µg/mL biotinylated detection antibody followed by a 30-minute incubation with peroxidase-conjugated streptavidin (Zymed, San Francisco, Calif) or with 2.6 µg/mL peroxidase-conjugated antihuman IgE, respectively. After development with 2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) substrate (Sigma) the plates were read at 405 nm in a microplate ELISA reader model 450 (Bio-Rad, München, Germany) with use of the microplate manager software (Bio-Rad). Cytokine and Ig concentrations were calculated according to the standard curves. The detection limit was 8 pg/mL for IL-4, 32 pg/mL for all other cytokines, and 1 ng/mL for IgE.

Statistics

The Student *t* test was used to test the statistical significance of the results; *P* ≤ .05 was considered significant.

RESULTS

DCs from atopic and nonatopic individuals do not differ in their phenotype and stimulate proliferation of autologous CD45RA⁺ and CD45R0⁺ CD4⁺ T cells equally efficiently

First, the phenotype and the proliferation-inducing capacity of mature DCs derived from CD14⁺ monocytes from atopic and nonatopic donors were analyzed. In Fig 1 there is shown that mature DCs from atopic donors demonstrated the same microscopic morphologic features as DCs from nonatopic donors (*A*) and express equal levels of MHC class II, the costimulatory molecules CD80 and CD86, and the DC-specific marker CD83 compared with DCs from nonatopic donors (*B*). Furthermore, DCs from atopic donors were as effective as DCs from nonatopic donors in inducing T helper cell proliferation (Fig 2). The allergen-specific proliferative response of memory CD45R0⁺CD4⁺ T cells from both groups was about 2-fold higher than allergen-specific proliferation of naive CD45RA⁺CD4⁺ T cells. With use of unpulsed DCs as control cells, no activation of T cells was observed (Fig 2).

Induction of a T_H2 cytokine profile in memory and naive T helper cells from atopic but not from nonatopic donors after stimulation with autologous allergen-pulsed DCs

Next we studied the cytokine pattern of CD45RA⁺ and CD45R0⁺CD4⁺ T cells cocultured with autologous mature DCs, which have been pulsed with the "relevant" allergen to which an allergic immune response is detectable (positive skin test reactions and allergen-specific IgE in the sera). To achieve measurable amounts of cytokine production, T cells were restimulated twice with newly generated autologous allergen-pulsed DCs in a 7-day interval. As shown in Fig 3, naive (CD45RA⁺) (*A*) as well as memory (CD45R0⁺) CD4⁺ T cells (*B*) from atopic persons produced significantly higher amounts of

the T_H2 cytokines IL-4, IL-5, and IL-10 compared with T cells from nonatopic persons, whereas the production of the T_H1 cytokine IFN-γ was slightly but not significantly decreased in the atopic group. The increase in T_H2 cytokine production was especially strong in memory CD4⁺ T cells and was less pronounced in naive T helper cells. Unlike IL-5 and IL-10, which were expressed by T cells from all donors, IL-4 was only detectable in 7 of 20 nonatopic controls, but it was induced in 22 of the 24 examined atopic patients. With unpulsed DCs used as a control, production of all cytokines was very low or sometimes even not detectable (data not shown).

To analyze whether the described findings relate to the allergic sensitivities of the atopic donors, we used DCs pulsed with an irrelevant allergen (no allergen-specific IgE in the serum and no positive skin test reactions) for T-cell stimulation assays. In these experiments IL-4 and IL-5, but not IL-10, production of naive and memory CD4⁺ T cells was significantly reduced. However, the amounts of IL-4, IL-5, and IL-10 produced by memory CD4⁺ T cells from atopic donors were still higher than the levels from nonatopic persons (Fig 3, *A* and *B*). Additionally, the use of DCs pulsed with the antigen tetanus toxoid also demonstrated an enhanced production of T_H2 cytokines by T cells from the atopic group. But in contrast to allergens, tetanus toxoid induced a much stronger IFN-γ production (ratio IFN-IL-4 was 1.6 for CD45RA⁺ and 7.6 for CD45R0⁺CD4⁺ T cells) (Fig 3, *A* and *B*).

Allergen-pulsed DCs enhance IgE production of B cells from atopic donors

To assess whether the DC-induced production of T_H2 cytokines in naive and memory CD4⁺ T cells from atopic donors would correlate with increased IgE production, purified CD19⁺ B cells were cultured together with CD45RA⁺ or CD45R0⁺CD4⁺ T cells in the presence of exogenous IL-4 and irradiated CD40L-transfected L cells or CD32 L cells as control with or without the addition of unpulsed or allergen-pulsed DCs, and IgE production was measured after 12 days of culture. The addition of allergen-pulsed DCs enhanced the production of IgE by B cells stimulated with IL-4 and CD40L-transfected L cells, which was especially pronounced in the presence of memory T helper cells, whereas in the presence of naive T helper cells unpulsed DCs were almost as effective as allergen-pulsed DC in inducing IgE production (Fig 4). Little or no IgE production was detected with use of IL-4 and CD32-transfected L cells for B-cell stimulation as control. Unfortunately, allergen-specific IgE production could not be measured in this experimental *in vitro* system because of lack of sensitivity.

IL-12 production by DCs from atopic donors is not impaired and addition of anti-IL-12 does not enhance T_H2 cytokine production

To test whether DCs from atopic donors produce lower amounts of bioactive IL-12 and therefore may favor the production of T_H2 cytokines, we analyzed the cytokines

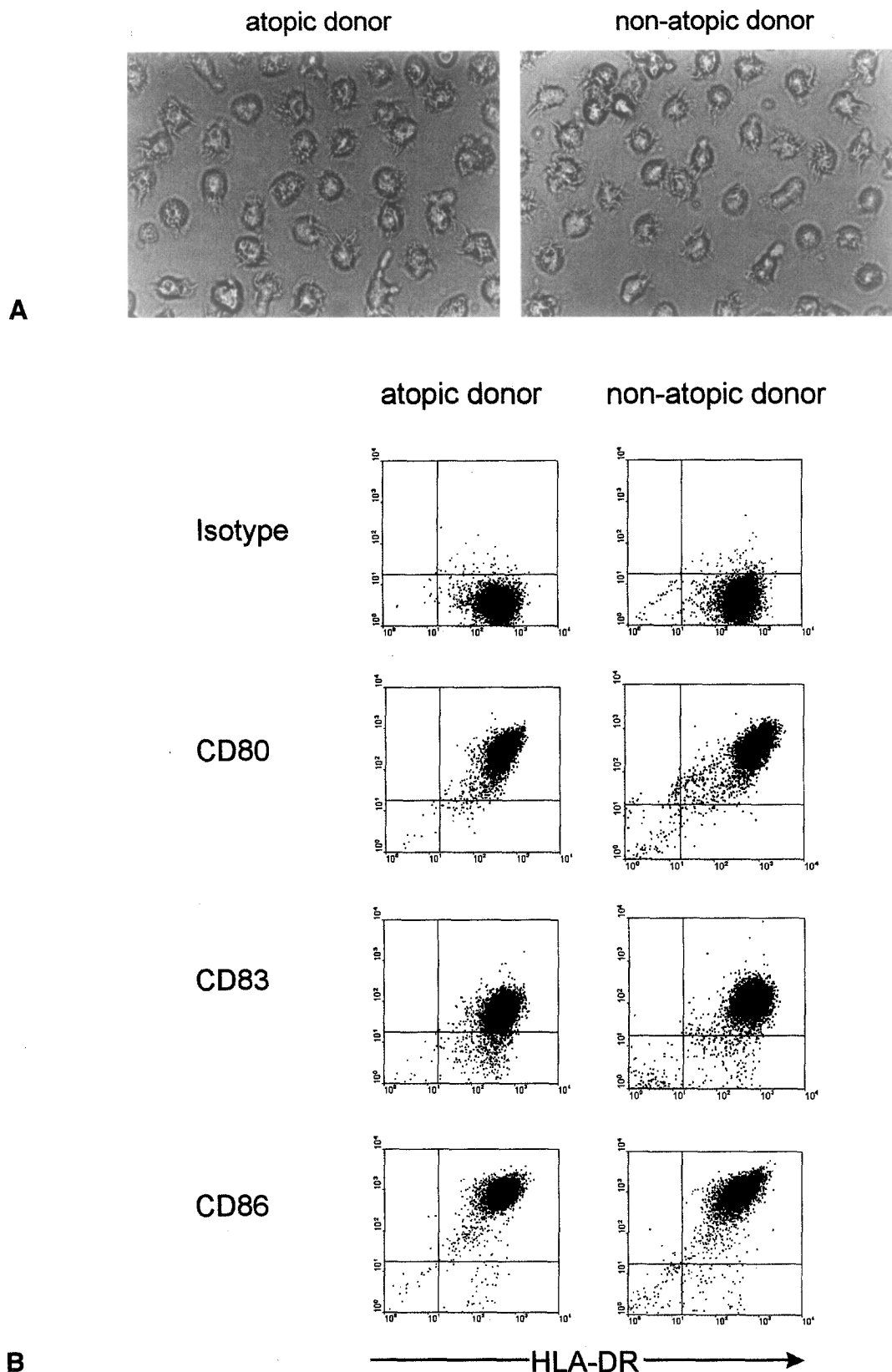


FIG 1. Comparison of phenotype of fully matured DCs from atopic and nonatopic donors. **A**, Cells were photographed and **(B)** double stained for HLA-DR and additional marker as indicated after 9 days of culture. The figure shows a representative example of 11 atopic and 10 nonatopic donors.

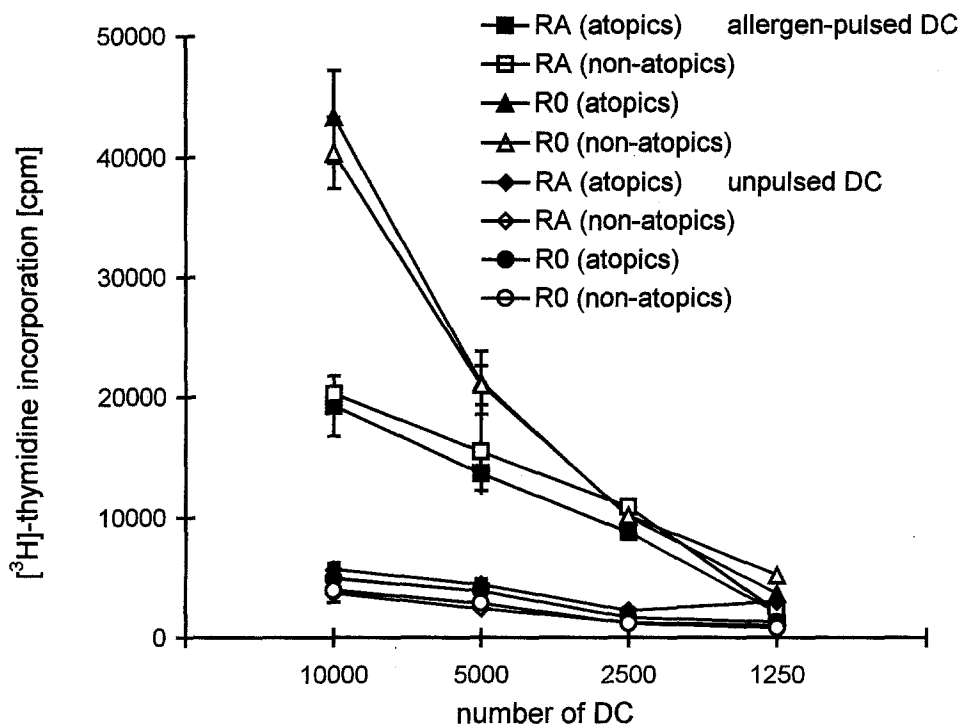


FIG 2. Comparison of the proliferative response of naive and memory T helper cells from atopic and nonatopic donors after stimulation with autologous allergen-pulsed DCs. A total of 1×10^5 CD45RA⁺ or CD45R0⁺CD4⁺ T cells were stimulated with titrated numbers of autologous allergen-pulsed or unpulsed DC. After 4 days the cells were pulsed with 1 μ Ci of [³H]TdR for 6 hours before harvesting. The data represent the mean $\pm$ SD from 4 independent experiments.

produced by mature DCs 24 to 48 hours after stimulation with allergens in the presence of IL-1 β , TNF- α , and PGE₂. In Fig 5 it is demonstrated that there was no difference between DCs derived from atopic donors compared with those from nonatopic donors concerning the production of bioactive IL-12p70 or the production of IL-12p40. Besides IL-12, we measured the secretion of the immunoregulatory cytokines IL-6, IL-8, IL-10, and MIP-1 α , which also play an important role in T-cell differentiation. Again, no differences could be observed between DCs derived from atopic or nonatopic subjects (Fig 5).

In consecutive experiments we investigated the effect of neutralizing antibodies against IL-12. Addition of anti-IL-12 mAb during the maturation phase of the DCs from days 7 to 9 or during the coculture of DCs and CD4⁺ T cells from atopic donors significantly diminished the production of IFN- γ but did not enhance the production of IL-4, IL-5, or IL-10 (Fig 6).

DISCUSSION

In the current study we have demonstrated that mature allergen-pulsed DCs are able to induce T_H2 cytokines, resulting in enhanced IgE production, in naive CD45RA⁺ and memory CD45R0⁺CD4⁺ T cells if the T cells were derived from atopic donors. The induction of T_H2 cytokines is not due to a diminished production of bioactive IL-12 by DCs from atopic individuals because they

produce equal amounts of IL-12p40 and IL-12p70 compared with DCs from nonatopic control subjects. Additionally, there is no difference in phenotype or T-cell stimulatory capacity by DCs derived from atopic or nonatopic persons. However, it cannot be excluded that there are differences between DCs from atopic and nonatopic donors in vivo that are no longer detectable after application of the culture methods described in Methods. Phenotypic differences of monocyte-derived DCs from atopic and nonatopic subjects have been described for the high-affinity receptor for IgE and CD11b, which were not analyzed in our study, but also for the expression of HLA-DR as well as an enhanced accessory potential of DCs from atopic patients in the mixed leukocyte reaction.²³ One reason for this discrepancy may be the fact that in the study of van den Heuvel et al²³ immature DCs were used for allergen presentation, whereas in our study fully matured DCs were used. Furthermore, mature DCs have been described to down-regulate the expression of their membrane-associated Fc γ R and Fc ϵ RI.²⁴

Our results concerning the induction of T_H2 cytokines by DCs are in line with several studies showing that DCs can also promote type 2 immune responses and consequently IgE production.^{18,19,25,26} The addition of PGE₂ together with proinflammatory cytokines on day 7 of DC culture to induce their final maturation may be one reason for this because it was demonstrated that PGE₂ inhibits IL-12 production by DCs and therefore favors the devel-

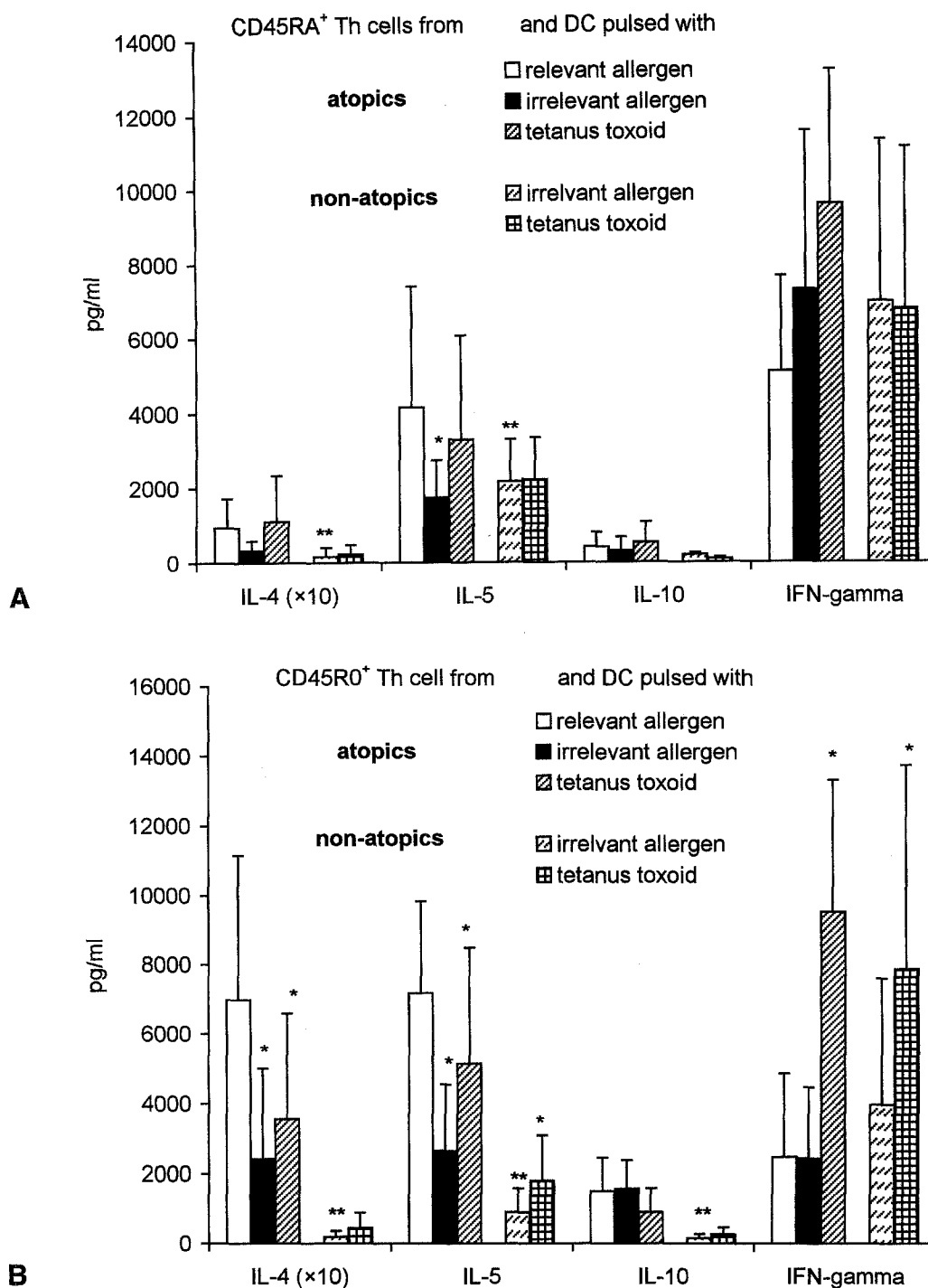


FIG 3. Comparison of cytokine production by naive and memory T helper cells from atopic and nonatopic donors after stimulation with autologous allergen- or tetanus toxoid-pulsed DCs. A total of 5×10^5 CD45RA⁺ (**A**) or CD45R0⁺ (**B**) CD4⁺ T cells were stimulated with 5×10^4 autologous DCs pulsed with a "relevant" allergen (ie, an allergen to which a pre-existing allergen-specific immune response is detectable [positive skin test or RAST >2]) or an "irrelevant" allergen (negative skin test or RAST <0.35), or tetanus toxoid. After 1 and 2 weeks of culture T cells were restimulated with 5×10^4 newly generated DCs. Supernatants were collected 24 hours after the second restimulation and assessed for IL-4, IL-5, IL-10, and IFN- γ content by ELISA. Results are expressed as the mean $\pm$ SD from 16 atopic and 15 nonatopic donors. Two asterisks, Statistically relevant differences ($P \leq .01$) between atopic and nonatopic subjects; asterisk, statistically relevant differences ($P \leq .05$) between DCs pulsed with a "relevant" allergen and DCs pulsed with an "irrelevant" allergen or tetanus toxoid (the label IL-4 $\times 10$ means that the production of IL-4 was multiplied by the factor 10 to avoid a presentation on 2 y axes).

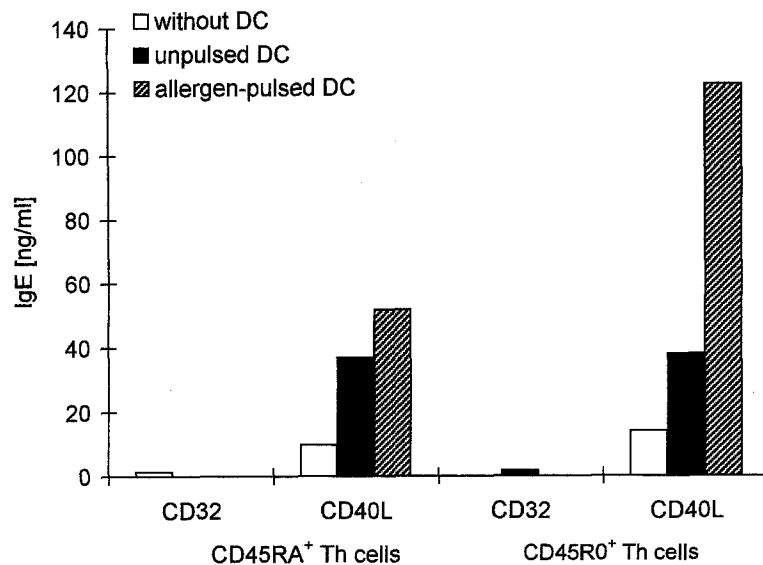


FIG 4. IgE production of B cells after stimulation with autologous T cells and allergen-pulsed DCs of atopic donors. A total of 5×10^5 CD45RA⁺ or CD45R0⁺CD4⁺ T cells were cocultured with 1×10^5 CD19⁺ B cells and stimulated with 5×10^4 autologous allergen-pulsed or unpulsed DCs in the presence of 1000 U/mL IL-4 and 2.5×10^4 irradiated CD40L L cells or CD32 L cells used as control. Supernatants were harvested after 12 days of culture and assessed for IgE content by ELISA. Shown is one representative of 8 independent experiments.

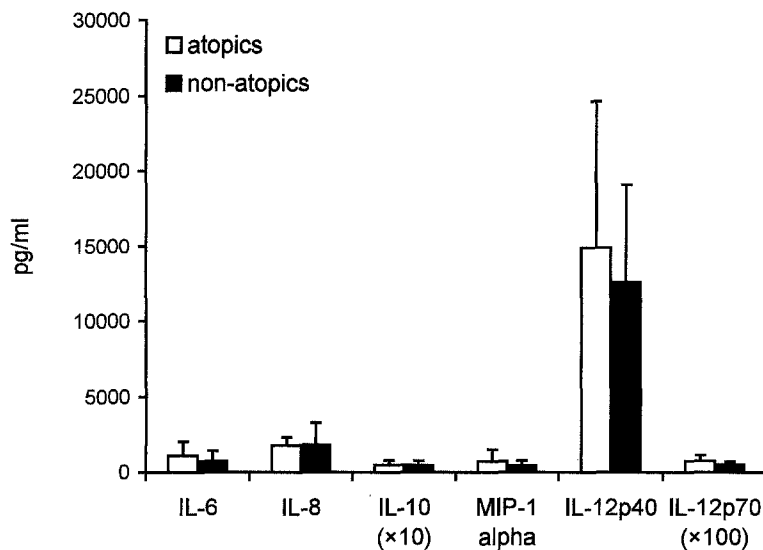


FIG 5. Comparison of cytokine production of DCs stimulated with allergen from atopic or nonatopic donors. DCs were generated from peripheral blood monocytes isolated from atopic or nonatopic donors. After 7 days of culture, immature DCs were pulsed with the respective allergen and stimulated with IL-1 β , TNF- α , and PGE₂ to induce full maturation. Supernatants were harvested 24 hours later for assessment of IL-12p40 and IL-12p70 production and after 48 hours for analysis of IL-6, IL-8, IL-10, and MIP-1 α production by ELISA. The results represent the means $\pm$ SD from 15 atopic and 13 nonatopic donors.

opment of T_H2 cells.¹⁸ In our study, however, DCs generated in the absence of PGE₂ induced lower amounts of IL-4 and IL-5 but also less IFN- γ (data not shown). Previously, Jonuleit et al¹⁶ demonstrated an enhanced production of IFN- γ by naive allogeneic T cells after stimulation with PGE₂-matured DCs. These discrepancies may be due to different conditions for DC culture (FCS-free media used in the study of Jonuleit et al and in our study versus FCS-containing media used in the study of

Kalinski et al) but also due to different conditions for induction of cytokines in naive T helper cells because in our study allergen-pulsed autologous DCs were used for primary, secondary, and tertiary stimulation whereas in the study of Kalinski et al naive T helper cells were initially activated by autologous DCs in the presence of the superantigen staphylococcal enterotoxin B, expanded with IL-2, and then restimulated unspecifically with anti-CD3 and anti-CD28 mAbs.¹⁸ Additionally, the enhanced

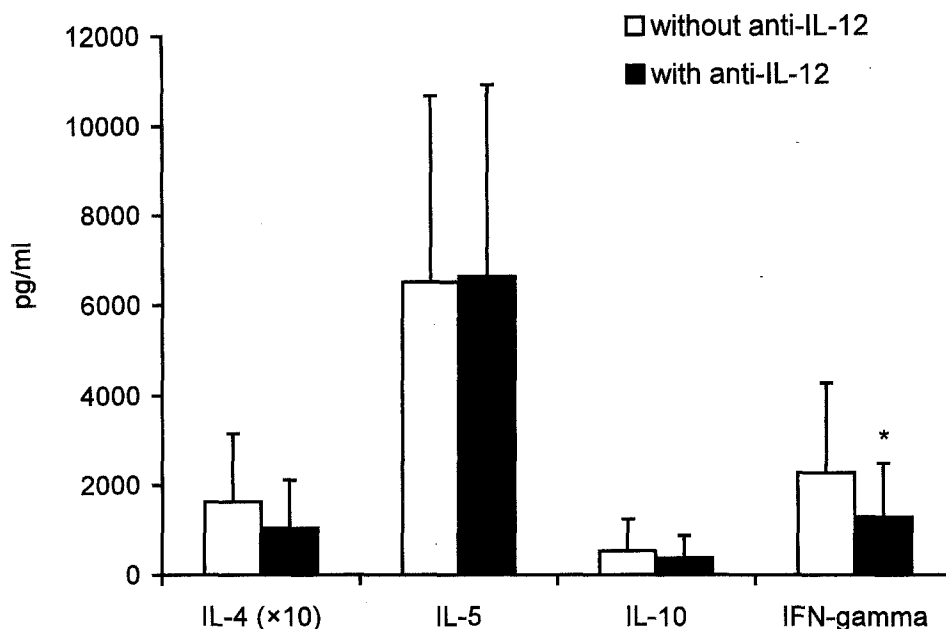


FIG 6. Effect of blocking IL-12 on cytokine production of T cells from atopic donors stimulated with autologous allergen-pulsed DCs. A total of 5×10^5 CD4⁺ T cells were stimulated with 5×10^4 autologous allergen-pulsed DCs generated with or without addition of neutralizing anti-IL-12 mAb during their maturation phase from days 7 to 9. After 1 week of culture, which was additionally performed in the presence or absence of anti-IL-12 mAb, T cells were restimulated with 5×10^4 newly generated DCs or anti-IL-12-treated DC. Supernatants were collected 24 hours later and assessed for IL-4, IL-5, IL-10, and IFN- γ content by ELISA. Shown are the means $\pm$ SD from 15 atopic donors. Asterisk, Statistically significant differences ($P \leq .05$) between untreated or anti-IL-12-treated DCs.

T_H2 cytokine pattern produced by naive and especially by memory T helper cells from atopic but not from nonatopic donors was observed despite production of equal amounts of IL-12 by fully matured DCs derived from atopic or nonatopic subjects. Furthermore, the addition of neutralizing anti-IL-12 mAb during the maturation phase of DCs and during coculture diminished IFN- γ but did not further increase IL-4 or IL-5 production.

Thus reasons other than diminished IL-12 production by DCs may explain our findings in this in vitro culture system, most likely the inherent property of T helper cells from atopic donors to preferentially produce cytokines of the T_H2 type, consequently leading to IgE production by B cells, which was especially seen for the memory T helper cell population but also detectable in naive T helper cells.^{27,28} This presumption is further supported by the fact that IL-4 production was not detectable in 65% of the examined cell cultures from nonatopic persons, although it was produced by 92% of the cultures from atopic patients. Furthermore, DCs pulsed with an "irrelevant" allergen (an allergen, to which no pre-existing allergic immune response could be demonstrated) or with tetanus toxoid still induced increased levels of T_H2 cytokines in T helper cells from the atopic group. In these experiments, however, the IFN- γ /IL-4 and IFN- γ /IL-5 ratios were higher compared with the ratio induced by DCs pulsed with the relevant allergen, indicating that the allergens to which the atopic donors were already sensitized had an especially high potency to induce T_H2-type responses, resulting in allergic diseases of the immediate type in these donors.

Culture systems such as the one presented here are useful for the analysis of human allergic immune responses in vitro, including the induction of naive T-cell responses when DCs are used. Such cell cultures may also be useful for diagnostic purposes, when memory T cells are analyzed. Similar assays may support the diagnosis of cellular immune responses underlying allergic reactions of the delayed type. Additional studies are needed to delineate definitively whether the preferential development of T_H2 cells is an intrinsic property of the T cells from atopic donors or whether it is rather influenced by antigen-presenting cells or other factors. It appears not unlikely that different cell types contribute to the development of the atopic/allergic phenotype and that the contribution of each cell type or factor may vary from one atopic individual to another. Our study suggests that the T cell plays a critical role in this respect.

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