

Increase of dendritic cells of type 2 (DC2) by altered response to IL-4 in atopic patients

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Background: Recent studies have shown that there are 2 dendritic cell subpopulations, DC1 and DC2, which induce T_H1 and T_H2 cell differentiation in vitro, respectively.

Objective: The purpose of this study was to determine whether there exists a deviation of DC1 and DC2 subsets and to investigate their functional abnormalities in T_H2 cell-mediated atopic diseases.

Methods: We analyzed the frequencies of DC1 (CD11c⁺CD123⁻) and DC2 (CD11c⁺CD123⁺) cells in peripheral blood of atopic patients; we also studied the responses of DC2 cells from atopic patients to IL-3 and IL-4 for their survival.

Results: DC2 cells but not DC1 cells were significantly increased in peripheral blood of atopic patients in comparison with that of healthy subjects. DC2 cell numbers were positively correlated with serum IgE levels and blood eosinophil counts, the increase of which reflects T_H2-type immune response in atopic diseases. IL-4 inhibited IL-3-induced survival of DC2 cells from healthy controls, but IL-4 failed to suppress the IL-3-induced survival of DC2 cells from atopic patients. Furthermore, IL-4 alone enhanced the survival of DC2 cells from atopic patients but not from healthy controls. However, no significant differences were found in the expression levels of activation/maturation markers on DC2 cells between atopic patients and healthy controls.

Conclusion: These results indicate that DC2 cells are preferentially increased in atopic patients in correlation with the state of atopic allergy and that DC2 cells in atopic patients, unlike those in healthy subjects, exhibit altered responses to IL-4 for survival, suggesting that DC2 cells in atopic patients might contribute to the enhanced T_H2 cell differentiation in atopic diseases. (*J Allergy Clin Immunol* 2001;108:1005-11.)

Key words: Dendritic cells, DC1, DC2, atopy, T_H2, IL-4

Abbreviations used

DC: Dendritic cell
DC1: Dendritic cell type 1
DC2: Dendritic cell type 2

A considerable amount of investigation has centered on the pathogenesis of atopic disorders, including asthma and atopic dermatitis, which are characterized by IgE production and eosinophilic inflammation. The discovery of T_H1 and T_H2 subsets of CD4⁺ T cells has helped to explain the immunologic basis for the diversity of T cell responses; T_H1 cells produce IL-2, IFN- γ , and lymphotoxin, whereas T_H2 cells produce IL-4, IL-5, IL-10, and IL-13.¹⁻⁴ Accordingly, cytokine production in atopic disorders is known to exhibit a bias toward the T_H2 cytokines, IL-4 and IL-5,⁵⁻⁸ and the requirement of these cytokines has been confirmed through use of murine models of allergic inflammation.⁹⁻¹¹ However, the mechanisms that lead to T_H2 effector cell differentiation in atopic disorders are poorly understood.

Dendritic cells (DCs) are the most efficient antigen-presenting cells (APCs) that can prime naive T cells to antigens.^{12,13} Moreover, DCs can influence the differentiation of naive T cells into T_H1 or T_H2 effector cells.^{14,15} Recent studies have shown that there are 2 distinct lineages of human DCs: myeloid DCs and lymphoid DCs.^{12,13,15,16}

Myeloid DCs (DC1) are CD11c⁺, express other myeloid markers (CD13 and CD33), and require GM-CSF for their survival.^{14,17-19} Mature DC1 cells produce large amounts of IL-12 and induce T_H1 cell differentiation.^{14,20,21}

Lymphoid DCs (DC2) have been described as plasmacytoid cells in peripheral tissues, are CD11c⁻, express IL-3 receptor α chain (CD123), and require IL-3 but not GM-CSF for their survival and maturation.^{14,17,18,22} Mature DC2 cells can induce T_H2 cell differentiation through IL-4-independent mechanisms.^{14,23,24} Furthermore, a negative feedback control of T-helper cells and dendritic cell subsets has been reported: T_H2 cytokine IL-4 inhibits the survival of DC2 cells,^{14,18} whereas T_H1 cytokine IFN- γ rescues DC2 cell death induced by IL-4.¹⁴ However, the role of DC1 and DC2 cells in T_H2 cell-mediated atopic diseases has not yet been clarified.

To determine whether there exists a deviation of DC1

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Supported in part by grants from the Ministry of Education, Science and Culture and from the Ministry of Health and Welfare, Japan.

Received for publication May 21, 2001; revised August 17, 2001; accepted for publication August 21, 2001.

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0091-6749/2001 \$35.00 + 0 1/84/119741

doi:10.1067/mai.2001.119741

TABLE I. Profiles of atopic patients studied

Group	No. of patients	Age (y)	Sex (M/F)	Serum IgE	Blood eosinophils (/mm ³)
Atopic asthma	12	27.0 ± 4.2*	6/6	2392 ± 2573*	473 ± 312*
Atopic dermatitis	16	25.1 ± 7.1	7/9	3714 ± 3120	605 ± 315
Healthy control	20	26.8 ± 3.4	11/9	59 ± 21	91 ± 72

M/F, Male/female.

*Mean ± SD.

and DC2 subsets and to investigate their functional abnormalities in patients with atopic diseases, we analyzed the frequencies of DC1 and DC2 cells in peripheral blood of patients with atopic asthma and atopic dermatitis by means of flow cytometry, and we examined the relationship with serum IgE levels and blood eosinophil counts. We also studied the responses of DC2 cells from atopic patients to IL-3 and IL-4 for their survival, because it has been shown that the survival and death of DC2 cells is regulated by IL-3 and IL-4.^{14,18,22} Our results indicate that DC2 cells are preferentially increased in atopic patients in correlation with the state of atopic allergy and that DC2 cells in atopic patients, unlike those in healthy subjects, survive in response to IL-4. These results suggest that DC2 cells in atopic patients might thus contribute to the enhanced T_H2 cell differentiation in atopic diseases.

METHODS

Patients

Twelve patients with atopic asthma²⁵ and 16 patients with atopic dermatitis²⁶ were studied (Table I). All were atopic patients who had (1) greater than 300 U/mL of serum IgE, as determined with radioimmunosorbent testing,²⁷ and (2) positive RAST results against at least 1 of 30 allergens, including *Dermatophagoides farinae*, house dust, cat dander, grass pollens, and 5 molds. Asthmatic patients were taking inhaled β_2 -adrenergic agonists and/or oral theophyllines; some of them also took inhaled beclomethasone, but none took oral corticosteroids. Patients with atopic dermatitis were treated with corticosteroid ointments but no oral corticosteroids. All patients were in stable condition with respect to the disease when they were analyzed. Twenty healthy subjects with no history of atopic diseases and less than 100 U/mL of serum IgE were also examined as controls.

Flow cytometric analysis

PBMCs were isolated from heparinized venous blood of 28 atopic patients and 20 healthy subjects by Ficoll-Paque (Pharmacia Biotech Inc, Piscataway, NJ) density gradient centrifugation. Cells were stained with FITC-, PE-, PerCP-, or APC-conjugated mAbs to CD3, CD11c, CD14, CD16, CD19, CD20, CD34, CD56, CD123, and HLA-DR (Becton Dickinson, Mountain View, Calif) and analyzed on FACScalibur (Becton Dickinson) through use of CellQuest software. Dendritic cells were identified as cells positive for HLA-DR and negative for lineage markers on T cells (CD3), B cells (CD19, CD20), natural killer cells (CD16, CD56), monocytes (CD14), neutrophils (CD16), and hematopoietic progenitor cells (CD34).^{12-15,17,18} DC1 and DC2 subsets were identified as CD11c⁺CD123⁻ DCs and CD11c⁻CD123⁺ DCs, respectively.^{12-15,17,18} Positive cells for CD markers were defined as the cells that were stained with mAbs to respective CD markers at the fluorescent intensity greater than that of cells stained with their isotype-matched control mAbs. Selected samples were stained with PE-con-

jugated mAbs to CD4 (Becton Dickinson), CD40, CD80, CD86, and CD124 (Coulter, Fullerton, Calif).

Isolation of DC2 cells from PBMCs

PBMCs were stained with FITC-conjugated mAbs to lineage markers, including CD3, CD14, CD16, CD19, CD20, CD34, and CD56, in PBS containing 1% FCS at 4°C for 30 minutes. After washing with PBS, cells were incubated with anti-FITC magnetic microbeads (Miltenyi Biotec, Sunnyvale, Calif) at 4°C for 20 minutes, washed 3 times with PBS, and passed through the MACS separation BS column (Miltenyi Biotec) according to the manufacturer's instructions. Recovered cells were stained with anti-CD11c PE, washed with PBS, incubated with anti-PE magnetic microbeads (Miltenyi Biotec), and passed through the MACS separation BS column. The recovered cells were then incubated with anti-CD4 magnetic microbeads and positively selected on a magnetic separation column (Miltenyi Biotec). Cells were kept at 4°C during the entire procedure. The purity of cells was evaluated by FACS with antilineage markers FITC, anti-HLA-DR PerCP, anti-CD11c PE, and anti-CD123 APCs; it was consistently greater than 95% for DC2 cells.¹⁴ The yields of DC2 cells from 100 mL of peripheral blood were approximately 4 to 8 × 10⁴.

Effect of IL-3 and IL-4 on DC2 survival

To determine the effects of IL-3 and IL-4 on DC2 cell survival, isolated DC2 cells were cultured in triplicate at 37°C for 72 hours at 2 × 10⁴ per well in flat-bottomed microtiter plates in RPMI 1640 medium containing 10% FCS in the presence of 50 ng/mL of IL-3 (Genzyme, Cambridge, Mass) and/or 20 ng/mL or indicated doses of IL-4 (Genzyme). Cells were harvested and numbers of viable cells were determined by trypan blue dye exclusion through use of a hemocytometer. In some experiments, viability was also determined by FACS through use of 5 μ g/mL of propidium iodide (PI; Boehringer Mannheim, Indianapolis, Ind). To measure cell proliferation, cells were pulsed for the last 18 hours with 0.5 μ Ci of ³H-thymidine per well, and the incorporation of the radionucleotide was measured by means of a β -scintillation counter.

Data analysis

Data are summarized as means ± SDs. The statistical analysis of the results was performed through use of the unpaired *t* test. *P* values less than .05 were considered significant.

RESULTS

Increase of DC2 cells in peripheral blood of atopic patients

It has recently been shown that DC1 and DC2 subsets are importantly involved in inducing T-helper cell differentiation.^{14,15} To determine whether DC1 and DC2 cells are deviated in T_H2 cell-mediated atopic diseases, we first examined the number of DC1 and DC2 cells in peripheral blood of atopic patients by means of flow cytometry. Dendritic cells were identified as HLA-DR⁺

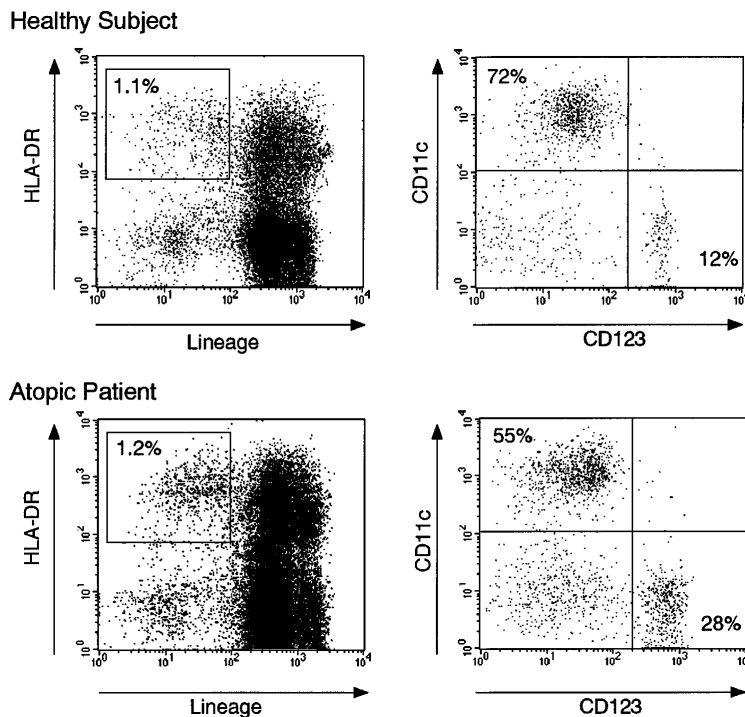


FIG 1. Detection of DC1 and DC2 subsets in peripheral blood of healthy subjects and atopic patients by means of flow cytometry. PBMCs were isolated from healthy subjects (*upper panels*) and atopic patients (*lower panels*). For 4-color flow cytometric analysis, cells were stained with anti-HLA-DR PerCP, antilineage markers (CD3, CD14, CD16, CD19, CD20, CD34, and CD56), FITC, anti-CD11c APCs, and anti-CD123 PE and analyzed on FACS. DCs were identified as HLA-DR⁺ lineage⁻ (CD3⁻, CD14⁻, CD16⁻, CD19⁻, CD20⁻, CD34⁻, and CD56⁻) cells (*left*); DC1 and DC2 cells were identified as CD11c⁺CD123⁻ DCs and CD11c⁻CD123⁺ DCs, respectively (*right*). Shown are representative FACS profiles from healthy subjects and atopic patients.

lineage⁻ (CD3⁻, CD14⁻, CD16⁻, CD19⁻, CD20⁻, CD34⁻, and CD56⁻) cells, and DC1 and DC2 cells were identified as CD11c⁺CD123⁻ DCs and CD11c⁻CD123⁺ DCs, respectively^{12-15,17,18} (Fig 1).

DC2 cells were significantly increased in peripheral blood of atopic patients in comparison with those of healthy subjects, whereas with respect to the number of DC1 cells atopic patients and healthy subjects were not significantly different. The numbers of dendritic cells (mean $\pm$ SD) in peripheral blood were similar in healthy subjects ($20,151 \pm 8,669/\text{mL}$; $n = 20$) and atopic patients ($22,872 \pm 8,002/\text{mL}$; $n = 28$). DC1 cells were also similar in number in atopic patients and healthy subjects ($14,449 \pm 6,390/\text{mL}$ vs $13,842 \pm 4,918/\text{mL}$; Fig 2, A). In contrast, DC2 cells were significantly increased in atopic patients ($4,506 \pm 2,136/\text{mL}$; $P < .001$) in comparison with healthy subjects ($2,192 \pm 998/\text{mL}$; Fig 2, A). Moreover, the DC2/DC1 ratio was significantly higher in atopic patients than in healthy subjects (0.387 ± 0.250 vs 0.162 ± 0.044 ; $P < .001$; Fig 2, B). In addition, serial determinations of DC1 and DC2 numbers at a 3- to 4-month interval in 6 atopic patients and 7 healthy subjects revealed that the variation of DC1 and DC2 numbers was small (7.5% and 10.9%, respectively) during that period and was thus a stable parameter. These results indicate that DC2 cells are preferentially increased in peripheral blood of atopic patients.

Correlation between DC2 cell numbers and IgE levels and eosinophil counts

To determine whether increased DC2 cells play a role in the development of atopic diseases, we examined the correlation between DC2 cell numbers and serum IgE levels and blood eosinophil counts. Serum IgE levels and blood eosinophil counts were determined at the time of the determination of DC1 and DC2 subsets. DC2 cell numbers were positively correlated with serum IgE levels ($r = 0.489$; $n = 48$; $P < .001$) and blood eosinophil counts ($r = 0.526$; $P < .001$; Fig 3). In addition, DC2/DC1 ratio was also correlated with serum IgE levels ($r = 0.475$; $P < .001$) and blood eosinophil counts ($r = 0.488$; $P < .001$). However, no significant correlation was found between DC1 cell numbers and IgE levels or eosinophil counts (data not shown). These findings of the positive correlation between DC2 cells and IgE levels and eosinophil counts suggest that DC2 cells might be involved in the development of T_H2 cell-mediated immune responses in atopic diseases.

IL-4 fails to suppress IL-3-induced survival of DC2 cells of atopic patients

It has been shown that the survival of DC2 cells is controlled by IL-3 and IL-4.^{14,18,22} IL-3 enhances survival of DC2 cells and induces maturation of these cells, where-

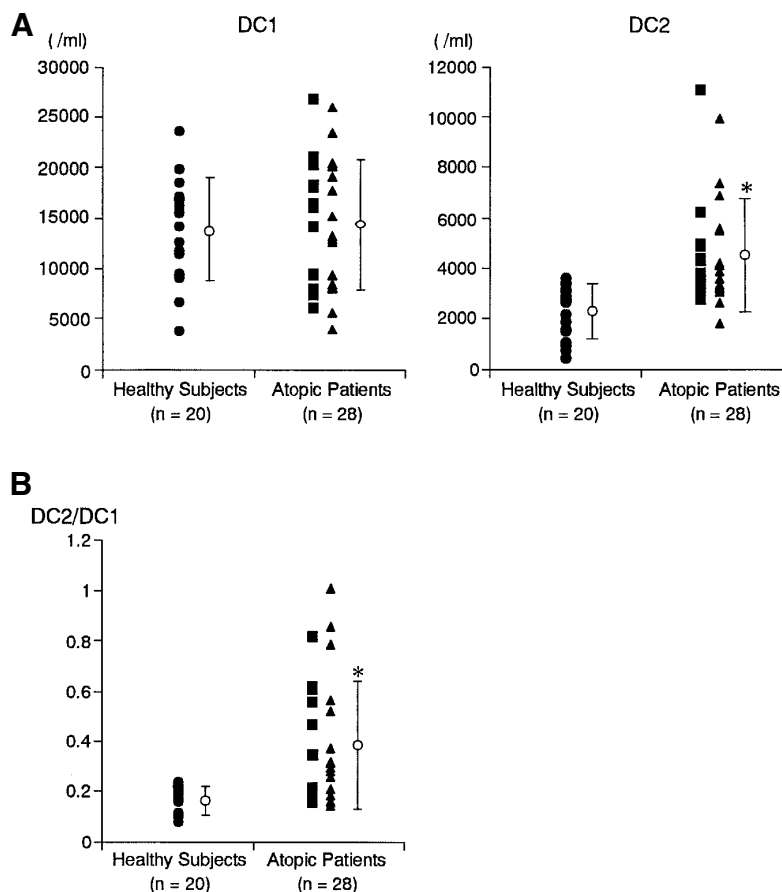


FIG 2. DC2 cells but not DC1 cells are increased in peripheral blood of atopic patients. DC1 and DC2 cell numbers in peripheral blood (**A**) and the DC2/DC1 ratio (**B**) were calculated from healthy subjects (n = 20; circles) and atopic patients (patients with atopic asthma [n = 12] and patients with atopic dermatitis [n = 16]; squares and triangles, respectively) on the basis of the numbers of PBMCs and DC1 and DC2 subsets by means of flow cytometry. Open circles and vertical lines indicate means $\pm$ SDs of the groups. *Significantly different from the mean value of healthy controls; $P < .001$.

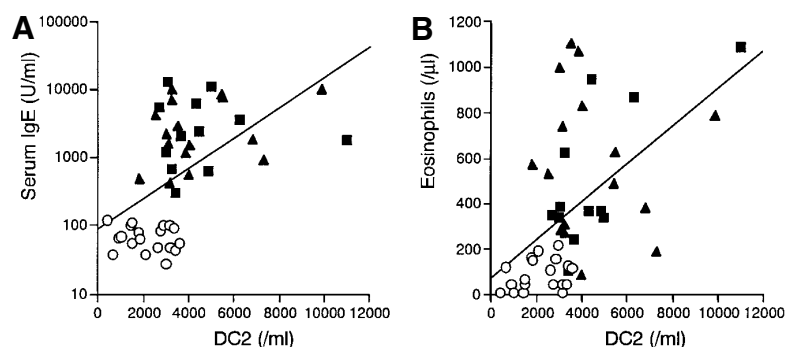


FIG 3. Correlation between DC2 cell numbers and serum IgE levels (**A**) and blood eosinophil counts (**B**). The numbers of DC2 cells in peripheral blood of healthy subjects (n = 20; open circles) and atopic patients (patients with atopic asthma [n = 12] and patients with atopic dermatitis [n = 16]; closed squares and closed triangles, respectively) were determined as described in the legend to Fig 2. DC2 cell numbers were significantly correlated with serum IgE levels ($r = 0.489$; $P < .001$) and blood eosinophil counts ($r = 0.526$; $P < .001$).

as IL-4 abrogates the IL-3-induced survival of DC2 cells.^{14,18,22} To determine whether the increase of DC2 cells in atopic patients is caused by the altered responses of atopic DC2 cells to IL-3 and IL-4 for their survival, we

examined the effect of IL-3 and IL-4 on survival of DC2 cells from atopic patients and healthy subjects.

IL-3 similarly enhanced survival of DC2 cells from both healthy subjects and atopic patients. In the absence

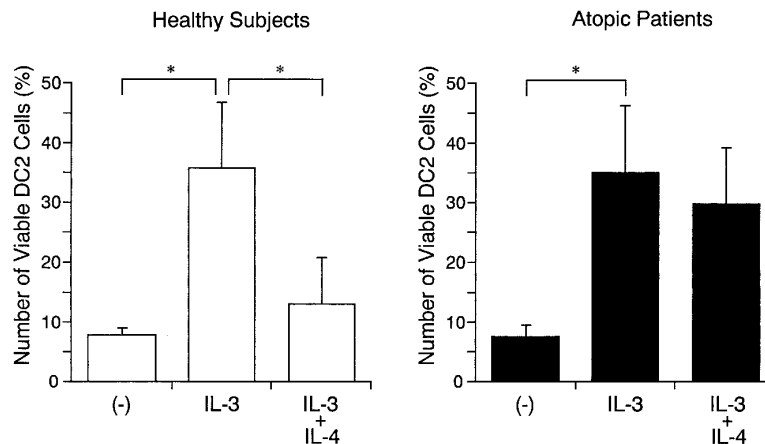


FIG 4. IL-4 fails to suppress IL-3-induced survival of DC2 cells in atopic patients but not in healthy subjects. DC2 cells were isolated from peripheral blood of healthy subjects ($n = 5$; left) and atopic patients ($n = 5$; right) by means of magnetic cell sorting, as described in the Methods section, and were cultured for 72 hours in the presence of IL-3 (50 ng/mL) and IL-4 (20 ng/mL). Cells were then harvested, and numbers of viable cells were determined by means of trypan blue dye exclusion. Data are means $\pm$ SDs in each group. $*P < .005$.

of IL-3, DC2 cells were rendered apoptosis, and only 7.2% and 7.3% of DC2 cells from healthy subjects ($n = 5$) and atopic patients ($n = 5$), respectively, were viable after 72 hours of culture (Fig 4). When cultured with IL-3 (50 ng/mL) for 72 hours, the survival of DC2 cells was significantly increased in both healthy subjects ($35.7\% \pm 10.9\%$; $n = 5$; $P < .005$) and atopic patients ($34.9\% \pm 11.3\%$; $n = 5$; $P < .005$; Fig 4). In addition, DC2 cells cultured with IL-3 differentiated to mature DC2 cells, as indicated by the morphologic changes and increased expression of CD80, CD86, and HLA-DR (data not shown). Therefore, DC2 cells from atopic patients and healthy subjects responded similarly to IL-3 to survive and differentiate to mature DC2 cells.

On the other hand, IL-4 failed to inhibit IL-3-induced survival of DC2 cells from atopic patients but not of DC2 cells from healthy subjects. IL-4 (20 ng/mL) significantly decreased IL-3-induced survival of DC2 cells from healthy subjects in comparison with those cultured with IL-3 alone ($12.8\% \pm 4.7\%$; $n = 5$; $P < .005$; Fig 4), which was consistent with previous reports.^{17,18,22} However, IL-4 could not significantly inhibit IL-3-induced survival of DC2 cells from atopic patients ($29.5\% \pm 9.3\%$; $n = 5$; Fig 4). These results indicate that to survive, DC2 cells of atopic patients respond differently to IL-4 than those of healthy subjects.

IL-4 enhances survival of DC2 cells of atopic patients but not that of healthy subjects

We then determined whether IL-4 increases the survival of DC2 cells from atopic patients. IL-4 (0.1–50 ng/mL) did not significantly affect the survival of DC2 cells from healthy subjects after 72 hours of culture (5% to 7% of viable cells; $n = 6$ at each dose; Fig 5). In contrast, IL-4 significantly increased the survival of DC2 cells from atopic patients up to 37% (at 10 ng/mL) in a dose-dependent manner ($P < .005$; $n = 6$; Fig 5). In addition, IL-4 inhibited the apoptosis of atopic DC2 cells

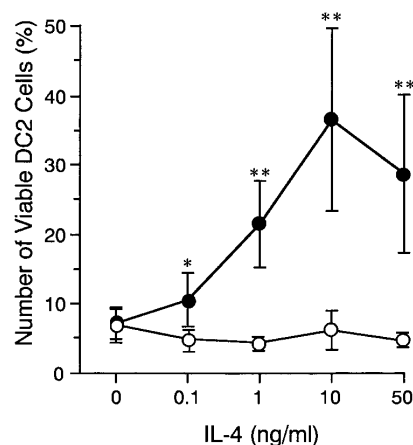


FIG 5. IL-4 enhances the survival of DC2 cells in atopic patients but not in healthy subjects. DC2 cells were isolated from peripheral blood of healthy subjects ($n = 6$; open circles) and atopic patients ($n = 6$; closed circles) by magnetic cell sorting, as described in the Methods section, and were cultured for 72 hours in the presence of IL-4 (0.1–50 ng/mL). Cells were then harvested, and numbers of viable cells were determined by means of trypan blue dye exclusion. Data are means $\pm$ SDs at each dose of IL-4. *Significantly different from the mean value of the survival without IL-4; $P < .05$. **Significantly different from the mean value of the survival without IL-4; $P < .005$.

detected by PI staining, and IL-4-stimulated atopic DC2 cells showed no significant increase of ^3H -thymidine incorporation (data not shown). However, the expression of IL-4 receptor α chain (CD124) on DC2 cells was not significantly different between healthy subjects and atopic patients (data not shown). These results indicate that IL-4 enhances the survival of DC2 cells in atopic patients but not in healthy subjects, suggesting that the altered response of atopic DC2 cells to IL-4 could contribute to the increase of DC2 cells in atopic diseases.

Activation states of DC2 cells from atopic patients

To assess the activation states of DC2 cells *in vivo*, we examined the expression levels of costimulatory molecules CD40, CD80, CD86, and HLA-DR on DC2 cells, which are upregulated by stimulation with cytokines such as IL-3 and TNF- α .^{18,22} Low levels of CD40 and no detectable levels of CD80 and CD86 were expressed on DC2 cells in peripheral blood of healthy subjects and atopic patients, and no significant differences were found in the expression levels of CD40, CD80, CD86, and HLA-DR on DC2 cells between healthy subjects and atopic patients (data not shown). It is thus suggested that there is no apparent difference in the activation states of DC2 cells *in vivo* between healthy subjects and atopic patients.

DISCUSSION

Human DCs constitute 2 subsets of DC1 and DC2 cells that differ in cell-surface phenotype, requirement for cytokines to survive and mature, and capacity to direct T-helper cell differentiation.^{12,13,15} In this study, we showed that DC2 cells are preferentially increased in atopic patients in correlation with the state of atopic allergy. We found that DC2 cells but not DC1 cells were increased in peripheral blood of patients with atopic diseases (Fig 2) and that DC2 cell numbers were positively correlated with serum IgE levels and blood eosinophil counts (Fig 3), the increase of which reflects T_H2-type immune response in atopic diseases.^{2,5-8} Moreover, we also showed that increased DC2 cells in atopic patients exhibit altered responses to IL-4 for survival. We found that IL-4 inhibited IL-3-induced survival of DC2 cells from healthy controls but that IL-4 failed to suppress the IL-3-induced survival of DC2 cells from atopic patients (Fig 4). Furthermore, we found that IL-4 alone enhanced the survival of DC2 cells from atopic patients but not of those from healthy controls (Fig 5). However, no significant differences were found in the expression levels of activation/maturation markers on DC2 cells between atopic patients and healthy controls. These results suggest that DC2 cells in atopic patients, unlike those in healthy subjects, survive and increase in response to IL-4 and thus might contribute to the enhanced T_H2 cell differentiation in atopic diseases.

Although the importance of DCs in immune regulation is widely accepted, the quantitative and functional abnormalities of DC1 and DC2 subsets in immunologic diseases, including atopic allergy and autoimmune diseases, have not as yet been clarified. We demonstrate here that there exists a preferential increase of DC2 cells, along with an altered response of these cells to IL-4 for the survival in atopic diseases. Given that *in vitro* studies have demonstrated that DC2 cells induce T_H2 cell differentiation of naive T cells,^{14,15,23,24} our results suggest that DC2 cells in atopic patients can be importantly involved in the development of T_H2 cell-mediated atopic diseases. Our findings of positive correlations of DC2 cells with IgE

levels and eosinophil counts, the markers of T_H2 response, in atopic patients also support this suggestion.

It has recently been shown that there exists a negative feedback regulation between T-helper cells and DC subsets.^{14,18} The T_H2 cytokine IL-4 counteracts IL-3-dependent survival of DC2 cells,^{14,18} and overproduction of IL-4 might thus inhibit the development of DC2 cells. In contrast, IL-4 enhances the maturation of DC1 cells in the presence of GM-CSF.^{14,18} These opposing effects of IL-4 on DC1 cells and DC2 cells might enhance T_H1 cell differentiation but might inhibit T_H2 cell differentiation. On the other hand, the T_H1 cytokine IFN- γ protects DC2 cells from IL-4-induced apoptosis and promotes DC2 cell development.¹⁴ However, we found that in contrast to healthy subjects, IL-4 in atopic patients failed to abrogate IL-3-induced survival of DC2 cells (Fig 4) and supported the survival of DC2 cells in the absence of IL-3 (Fig 5). Therefore, the loss of a negative feedback regulation by IL-4 might be involved in the increased DC2 cells and subsequently in the enhanced T_H2 cell differentiation in atopic patients.

IL-4 activates a number of signaling molecules, including Stat6 and PI3-kinase, and transduces proliferative and/or antiapoptotic signals in most cell types.²⁸ In contrast, IL-4 abrogates IL-3-induced survival of DC2 cells from healthy subjects (Fig 4).^{14,18,22} At present, the mechanisms by which IL-4 inhibits IL-3-dependent survival of DC2 cells from healthy subjects are unclear. In addition, the mechanisms by which IL-4 fails to inhibit IL-3-dependent survival of DC2 cells from atopic patients are also unknown. Recently, accumulating evidence has suggested that SOCS family proteins, which are induced by cytokine signaling, might be involved in the termination of cytokine signaling.²⁹ Among SOCS family members, SOCS-1 and SOCS-3 have been shown to suppress IL-4 signaling,^{30,31} whereas SOCS-2 and CIS upregulate IL-4 signaling.³¹ Therefore, the expression pattern of SOCS family proteins might be involved in opposing IL-4 effects on DC2 cells in healthy subjects and atopic patients. In addition, recent studies have shown that polymorphisms of IL-4 receptor α chain and a signaling molecule, Stat6, are associated with IL-4 responsiveness of B cells, including CD23 expression and IgE production in atopic diseases.³²⁻³⁴ These polymorphisms might also be involved in the abnormal IL-4 responsiveness of DC2 cells in atopic patients. These possibilities are now under investigation.

It has been shown that the death-promoting effect of IL-4 on DC2 cells depends on the maturation stage of DC2 cells because these cells progressively lose their sensitivity to IL-4 after 12 hours of culture with IL-3.¹⁸ Although we found that the activation/maturation state of DC2 cells was indistinguishable between atopic patients and healthy subjects, we cannot exclude the possibility that IL-3 and other cytokines induced the maturation of DC2 cells in atopic patients.

In conclusion, we have shown that DC2 cells, which are professional APCs for T_H2 cell differentiation, are preferentially increased in atopic patients in correlation with the atopic state and that DC2 cells in atopic patients, unlike those in healthy subjects, exhibit altered responses

es to IL-4 for survival. These results suggest that DC2 cells in atopic patients might contribute to the enhanced T_H2 cell differentiation in atopic diseases.

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