

Functional and phenotypic differences of monocyte-derived dendritic cells from allergic and nonallergic patients

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Background: Several studies suggest a role for dendritic cells in the pathogenesis of allergic disease.

Objective: The purpose of this study was to compare function and phenotype of monocyte-derived dendritic cells (MoDC) from allergic asthmatic patients and healthy control subjects.

Methods: MoDCs were developed by incubating adherent monocytes for 5 days with IL-4 and granulocyte-macrophage colony-stimulating factor. Phenotype was assessed with flow cytometry, and the antigen-presenting function was assessed with the allogeneic mixed leukocyte reaction and an autologous specific antigen presentation.

Results: The morphology of the MoDCs was characteristic for immature dendritic cells. MoDCs from allergic asthmatic patients showed phenotypic differences in the expression of HLA-DR, CD11b, and the high-affinity receptor for IgE. A clearly enhanced accessory potential of MoDCs from atopic asthmatic patients in the mixed leukocyte reaction was also shown. Moreover, house dust mite-specific T-cell proliferation was increased.

Conclusion: This study suggests the involvement of dendritic cells in the pathogenesis of atopic asthma by an increased immunostimulatory capacity of MoDCs. (*J Allergy Clin Immunol* 1998;101:90-5.)

Key words: Atopic disease, antigen presentation, asthma, *Candida albicans* antigen, CD11b, dendritic cells, HLA-DR, house dust mite allergen, immunoglobulin receptors, mixed leukocyte reaction, purified protein derivative

Dendritic cells (DCs) play a central role in antigen presentation.¹ DCs are also very potent stimulators of T-lymphocyte proliferation in an allogeneic mixed leukocyte reaction (MLR). Although DCs have discrete typical functional and phenotypic characteristics, the ontogeny of these cells is not completely understood.² Recently, an in vitro model has been developed to derive DCs from monocytes.³ Purified human peripheral blood monocytes cultured in the presence of granulocyte-

Abbreviations used

AA:	Atopic asthmatic patients
DC:	Dendritic cell
GM-CSF:	Granulocyte macrophage colony stimulating factor
HDM:	House dust mite
MLR:	Mixed leukocyte reaction
MoDC:	Monocyte-derived dendritic cell
NA:	Nonatopic control subjects
PI:	Proliferation index

macrophage colony-stimulating factor (GM-CSF) and IL-4 develop into cells that have characteristics of DCs and that process antigen with a high efficiency. This suggests that in vivo monocytes have the potential to become efficient antigen-presenting cells.³ The monocyte derived DC (MoDC) forms a good model for the in vivo immature DC.

Allergic diseases are characterized by local inflammation and elevated serum IgE levels with a specificity to allergens such as house dust mite (HDM), pollen, and animal danders. Virtually all patients with allergic asthma exhibit a chronic inflammation characterized by infiltrates of T lymphocytes and accessory cells with features of DCs.⁴ In the last decade, accumulating evidence has been presented that suggests a role for the T-helper lymphocyte in allergic inflammation.⁵ The question of why these allergen-specific T cells are activated has drawn attention to the role of antigen-presenting cells. In patients with asthma, the number of DCs in the airways is increased.⁶ Under some circumstances DCs preferentially activate type-2 T-helper cells.⁷ DCs also produce cytokines that can modulate the T-cell cytokine secretion pattern.⁸ We are especially interested in whether the suggested difference in antigen-presenting cells already exists on the level of the peripheral blood cells. To clarify the possible role of DCs in allergic disease, MoDCs from atopic and nonatopic individuals were phenotypically and functionally compared.

METHODS

Patients

Patients were selected on the basis of their history of asthma and allergy. Allergy was confirmed by skin tests and RASTs. The nonallergic (NA) control group consisted of nine patients,

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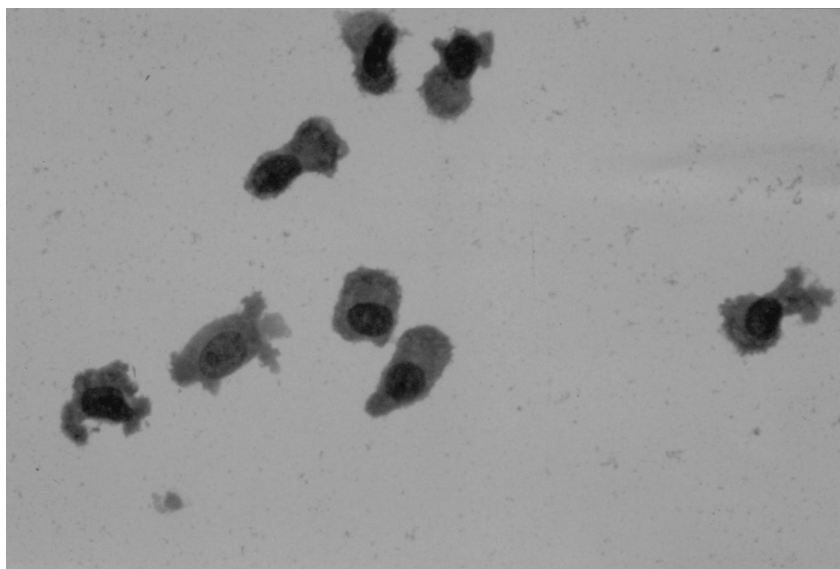


FIG. 1. MoDCs after 5-day culture with 500 U/ml IL-4 and 800 U/ml GM-CSF. MoDCs show eccentrically located nucleus and some blunt extending cell processes as is characteristic for immature DCs.

and the allergic asthmatic (AA) group consisted of eight volunteers. Bronchial hyperreactivity was evaluated by means of the histamine threshold (NA, >16 mg/ml; AA, 5.4 ± 2.3 mg/ml; $p < 0.000$).

Ethical approval

This study was approved by the institutional ethics committee. Informed consent was obtained from each subject before the study.

Media and reagents

The medium used was RPMI 1640 supplemented with 2 mmol/L L-glutamine, 10% heat-inactivated fetal calf serum (Gibco, Bio-Cult, Irvine, Scotland), 50 mmol/L β -mercaptoethanol, 50 U/ml penicillin, 100 μ g/ml streptomycin, and 50 μ g/ml gentamicin. In cultures of adherent monocytes, human recombinant IL-4 (rIL-4) (Genzyme, lot number B5665, Cambridge, Mass.) and rGM-CSF (Schering-Plough, Ireland) were added with a final concentration of 500 and 800 U/ml, respectively, to prepare the MoDCs.

For FACS analysis, the following monoclonal antibodies were used: Phycoerythrin-labeled CD1a (M-T102; PharMingen, San Diego, Calif.) (all other monoclonal antibodies were nonlabeled), CD1a (OKT6), CD3 (Leu4), CD4 (RIV6; Monosan, San Diego, Calif.) CD11b (OKM1), CD11c (p150-90; Dako, Glostrup, Denmark), CD14 (3C10), CD54 (K1, CLB, Amsterdam, The Netherlands), CD58 (15.2, CLB), CD83 (HB15a, kindly supplied by Dr. T. F. Tedder, Boston, Mass.),⁹ CD86 (FUN-1, PharMingen, San Diego, Calif.), HLA-DR/DQ (G3F10), RFD1 (kindly supplied by Dr. L. W. Poulter, London, U.K.),¹⁰ L25 (kindly supplied by Dr. T. Takami, Gifu, Japan), CD16 (Fc γ RIII, 3G8), CD32 (Fc γ RIIa, IV-3; panFc γ RII, AT10), CD64 (Fc γ RI, 22), CD89 (Fc α R, A77), CD23 (Fc ϵ RII, MHM6, Dako, Glostrup, Denmark), and Fc ϵ RI (H10). For isotype controls, mouse anti-rat antibodies were used with no known cross-reactivity to human antigens. They were ED8 (IgG1), ED3 (IgG2a), and HIS41 (IgG2b).

Cells

Monocytes and T lymphocytes were isolated from 30 ml heparinized blood. Mononuclear cells were isolated with a lymphoprep gradient (Nycomed, Oslo, Norway). Lymphocytes were isolated by rosetting to sheep red blood cells (Biotrading, Mijdrecht, The Netherlands). Monocytes were purified from the nonrosetting cells by adherence for 2 hours on petri dishes (B&D, Franklin Lakes, N.Y.). Nonadherent cells were removed by gently flushing with medium. Monocytes were cultured in medium containing 500 U/ml IL-4 and 800 U/ml GM-CSF. On day 5 the cells had become nonadherent and were collected. Viability was always above 95%. The morphology of the cells was determined with May-Grünwald Giemsa staining. The harvested cells proved to be a highly homogeneous population, containing only $10\% \pm 5\%$ other cell types (mostly CD3-positive T cells). These MoDCs were used for functional assays, as well as for the phenotypic analysis.

Allogeneic buffy coat T cells and autologous T cells were further purified by an adherence step of 2 hours on petri dishes. Nonadherent cells were layered on hypertonic 12.5% metrizamide, dissolved in medium (Nycomed, Oslo, Norway), and sedimented at $600 \times g$ for 10 minutes at room temperature. T cells were recovered from the pellet and washed twice. FACS analyses showed a purity greater than 95%. The autologous and allogeneic T cells were placed in aliquots (1.5 ml) and stored in liquid nitrogen until further use.

Mixed leukocyte reaction (MLR) assay

In all allogeneic MLR assays, buffy coat T cells from the same donor were used. T cells were isolated and purified as described above from a buffy coat and stored in liquid nitrogen (purity, 97.4%). On the day of use, T cells were thawed. Viability was always greater than 90%. Stimulator cells were irradiated (3000 rads) and added in graded doses to 1.0×10^5 T lymphocytes in 96-well U-bottom tissue culture plates (0.2 ml medium/well). T-cell proliferation was quantified by incubating with 1 μ Ci

TABLE I. Cell recovery during isolation and culture steps (average $\pm$ SEM)

	NAs	AAs	<i>p</i> Value
T cells	$19.7 \pm 7.9 \times 10^6$	$24.2 \pm 5.9 \times 10^6$	0.27
ER cells*	$9.6 \pm 1.7 \times 10^6$	$12.7 \pm 2.6 \times 10^6$	0.31
Recovery MoDC†	$2.8 \pm 0.4 \times 10^6$	$3.7 \pm 1.0 \times 10^6$	0.48

*ER cells are defined as the fraction of mononuclear cells not rosetting with neuraminidase-treated sheep erythrocytes. This is the monocyte-enriched fraction that will be further enriched by the plastic adherence step.

†Recovery of MoDCs after culture of adherent monocytes with IL-4 and GM-CSF.

(37kBq) of [methyl-³H] thymidine deoxyriboside (Amersham, Life Science, Buckinghamshire, U.K.) during the last 16 hours of the 4-day culture. Cells were harvested on a filter and radioactivity was measured in a liquid scintillation counter.

Specific antigen presentation assay

Autologous T cells were thawed on the day of use. Viability was always greater than 90%. MoDCs were incubated with 1.0×10^5 T cells in 96-well U-bottom tissue culture plates (0.2 ml medium/well) at a ratio of 1 MoDC to 40 T cells. The following antigens were added: *Candida albicans* antigen (ARTU biologicals, Lelystad, The Netherlands), purified protein derivative (Sanbio Uden, The Netherlands), and HDM antigen (ALK, Groningen, The Netherlands). T-cell proliferation in reaction to the antigens showed a plateau with concentrations between 1 and 20 μ g/ml. Therefore a final concentration of 10 μ g/ml was used for all antigens. T-cell proliferation was quantified by measuring the incorporated [methyl-³H] thymidine deoxyriboside (same method as above). The background T-cell proliferation was 1900 ± 200 cpm. No difference was found when the background levels between the different study groups were compared. The proliferation index (PI) was used for comparison of the experiments. The PI was calculated with the following formula:

$$PI = \frac{CPM(\text{MoDC} + \text{T cell} + \text{antigen})}{CPM(\text{MoDC} + \text{T cell})}$$

Flow cytometry

Cells were resuspended in phosphate-buffered saline with 0.1% bovine serum albumin and incubated with a panel of mouse anti-human monoclonal antibodies for 45 minutes at 4° C. Besides the panel of antibodies, isotype controls were used for each patient. Cells were washed twice and incubated for 45 minutes with fluorescein isothiocyanate-labeled F(ab')₂ fragments of goat anti-mouse antibody at 4° C. Cells were washed twice and incubated with Phycoerythrin-labeled mouse anti-human CD1a for 45 minutes at 4° C. Cells were washed and the fluorescence was measured on a FACScan flow cytometer (B&D). During analysis, cells were gated for CD1a positivity. The minimal positivity was used to determine the percentage of positive cells.¹¹

Statistical analysis

For statistical analysis, the paired Student's two-tailed *t* test was used for the comparison of allergic asthmatic patients with normal control subjects. A *p* value of < 0.05 was considered to indicate a significant difference between the groups.

TABLE II. Phenotypic characteristics of MoDCs (average $\pm$ SEM)

	NAs	AAs	<i>p</i> Value
Adhesion molecules			
CD11b	57.3 ± 6.9	76.6 ± 4.2	0.05
CD11c	42.0 ± 8.7	42.4 ± 15.9	0.98
CD54	73.7 ± 8.0	83.5 ± 14.8	0.38
CD58	28.3 ± 9.4	54.3 ± 7.5	0.06
Costimulatory molecules			
CD4	7.4 ± 2.3	10.7 ± 6.1	0.52
CD86	34.7 ± 6.0	32.9 ± 7.4	0.85
HLA-DR/DQ	73.7 ± 5.8	88.7 ± 2.2	0.04
Other phenotypic markers			
CD1a	49.5 ± 10.4	59.0 ± 12.0	0.55
CD14	19.6 ± 6.1	11.9 ± 3.9	0.33
CD83	20.9 ± 4.3	18.4 ± 7.2	0.77
L25*	75.7 ± 0.3	56.8 ± 16.6	0.44
RFD1	34.1 ± 10.2	30.7 ± 9.8	0.78
Immunoglobulin receptors			
FcγRI	10.4 ± 4.2	2.5 ± 1.0	0.15
FcγRIIpan	48.6 ± 7.4	57.4 ± 7.1	0.40
FcγRIIa	49.4 ± 6.6	55.9 ± 7.2	0.31
FcγRIII	12.6 ± 2.9	5.8 ± 1.7	0.09
FcαR	18.7 ± 1.9	12.0 ± 4.9	0.06
FcεRI	39.0 ± 6.7	16.8 ± 6.0	0.04
FcεRII	59.4 ± 5.8	71.9 ± 5.0	0.14

Percentage of positive cells $\pm$ SEM is shown.

*Group is too small for statistical comparison (*n* = 5).

RESULTS

Morphology

During the isolation procedure, the numbers of recovered T cells and nonrosetting cells were equal in both study groups (Table I). Also, the numbers of recovered MoDCs after culture with IL-4 and GM-CSF were comparable. The typical MoDC had an oval- or bean-shaped eccentrically located nucleus, with a basophilic cytoplasm and a few blunt extending cell processes (Fig. 1). No morphologic differences were found between the MoDCs from atopic and nonatopic patients.

Phenotypic analysis

Major histocompatibility complex class II was upregulated on MoDC from atopic subjects (Table II). Of all tested adhesion molecules, only CD11b differed significantly. CD58 expression was almost significantly increased in the AA group (*p* = 0.06). Of all tested Fc receptors, the expression was only found to differ significantly for the high-affinity receptor for IgE. However, there was also a tendency for CD16, CD64, and CD89 to be higher in the nonatopic group. In addition, another group of markers was studied with a less well-known function. A marked difference was found for the expression of L25, although the group was too small for statistical comparison (3 NA patients and 2 AA patients). CD14 expression was slightly higher on MoDCs from the control group, whereas CD1a expression was slightly lower. However, for both CD14 and CD1a the

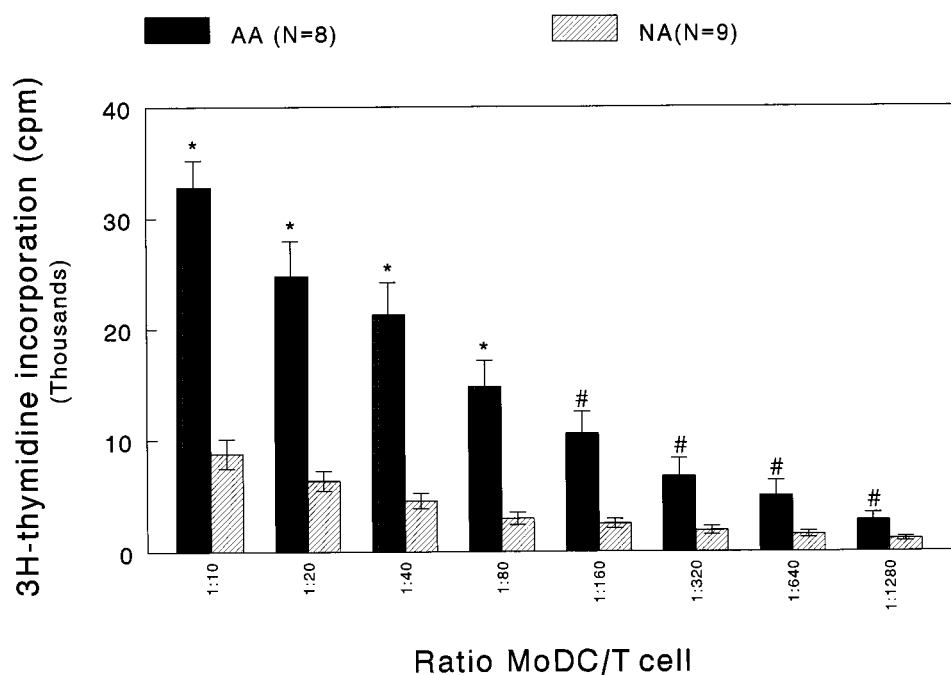


FIG. 2. Accessory potency of MoDCs in allogeneic mixed leukocyte reaction (average cpm $\pm$ SEM). MLR results after 4 days reveal marked difference between MoDC potency of atopic and nonatopic patients. Difference in accessory potency of MoDCs from AAs and NAs is highly significant from an MoDC/T cell ratio of 1:20 to 1:80. From 1:160 to 1:1280, difference remains statistically significant. * $p < 0.0005$. # $0.001 \leq p < 0.05$.

differences could not be confirmed statistically. No intensity differences were found (data not shown).

Functional analysis

MoDCs were found to be potent stimulators of T-cell proliferation in the allogeneic MLR (Fig. 2). These results confirm that MoDCs are very effective antigen-presenting cells. However, a highly significant difference was found when comparing MLR results between atopic subjects and NAs because MoDCs of the first group were a factor of 2 to 4 more potent at all tested ratios ($0.0005 \leq p < 0.05$). Because the used allogeneic T cells were all from the same buffy coat this difference can be totally attributed to differences in MoDC function.

MoDCs from all individuals were capable of presenting different antigens to autologous T cells (Fig. 3). The capacity to present irrelevant antigens (e.g., purified protein derivative and *Candida albicans* antigen) was not altered in the atopic group, whereas the response to HDM antigen in this group was increased ($p < 0.05$). This indicates that MoDCs from the atopic group are better at inducing the relevant allergen response (HDM) compared with the control group.

DISCUSSION

Several studies have shown functional and phenotypic differences between monocytes from atopic patients.^{12, 13} To our knowledge this is the first study that shows functional differences of DCs from atopic and nonatopic subjects. These results cannot be explained by "DC

purity." Even with a 10-fold difference in the number of DCs, the accessory potency of the MoDCs of nonatopic subjects would not reach the capacity of those of AAs. For instance, T-cell proliferation with an MoDC/T-cell ratio of 1:20 in nonatopic subjects was still lower than that of 1:320 found in AAs. Monocytes from atopic subjects develop into more potent accessory cells than those from normal control subjects, which indicates that there is a difference in MoDCs from atopic and control subjects. The enhanced accessory potency of the MoDCs from atopic patients might be explained by the increased expression of HLA-DR, CD11b, and CD58. The question remains whether these small differences are the only explanation. After all, other molecules that were found to be important in the MLR, such as CD54 and CD86, do not differ.¹⁴ Soluble mediators might be involved in the allogeneic MLR as well.

The mechanism behind the increased accessory potency of MoDCs from atopic subjects is unknown. Our data show that there is a tendency for a different expression of CD1a and CD14. This might suggest an altered sensitivity of the atopic monocytes for the cytokine IL-4 or GM-CSF. IL-4 is responsible for both the downregulation of CD14 and the increased accessory potency of monocytes.¹⁵ Studies on the IL-4 receptor expression or the IL-4 signaling pathway might provide an explanation for our results. The functional differences might also be explained by intrinsic properties of the monocytes of atopic patients, but previous systemic priming of the monocytes cannot be excluded.

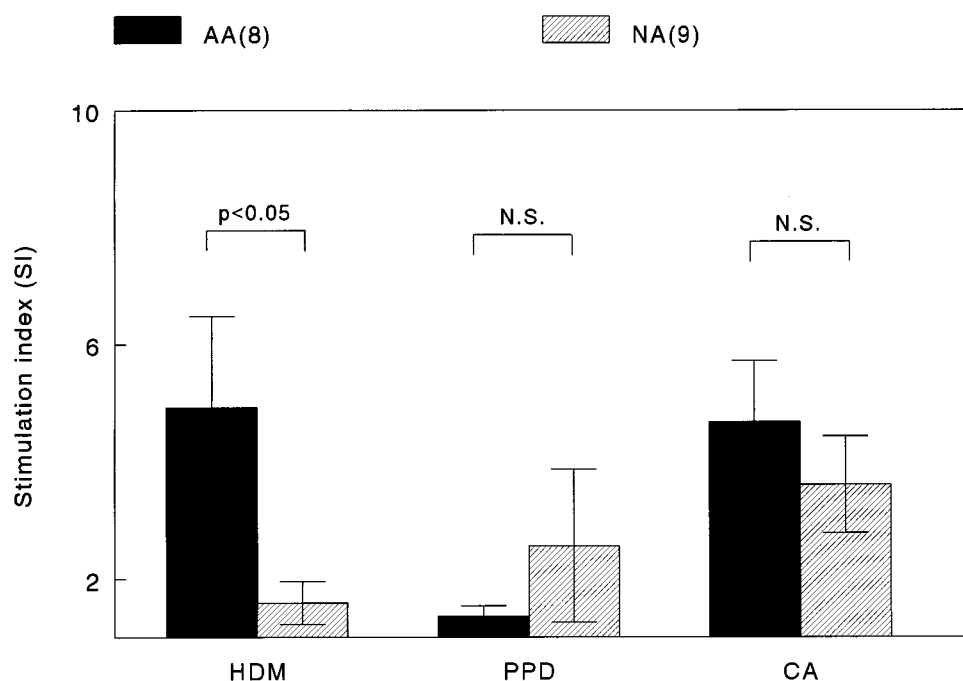


FIG. 3. Specific antigen presentation (average SI $\pm$ SEM). Antigen-presenting capacity was compared in a 4-day assay with an MoDC/T-cell ratio of 1:40. Comparing results of AAs with NAs, significantly different result is only found when HDM allergen is added. SI is calculated by dividing [^{3} H]-thymidine counts from the stimulator plus responder cells with antigen by counts from stimulator plus responder cells without antigen. N.S., Not significant; PPD, purified protein derivative; CA, *Candida albicans*.

In contrast to the increased accessory activity in the allogeneic MLR, the presentation of the *Candida albicans* antigen and purified protein derivative is not altered. However, T cells from atopic asthmatic individuals show a significantly higher proliferation in reaction to HDM antigen presentation. These results suggest that the capacity of MoDCs to process and present antigen in an immunoglobulin-independent manner is not altered in atopic individuals. The different reactivity to HDM allergen might be caused by the increased specific precursor T-cell population in atopic subjects.

However, we also show a difference in the immunoglobulin receptor expression on MoDCs. DCs carry receptors for different classes and isotype-specific immunoglobulin on the plasma membrane.^{16, 17, 18} Our study confirms these data and shows a discrete difference in the expression of high-affinity receptor for IgE on MoDCs when comparing atopic subjects with normal control subjects. The cytokine IL-4 is a known modulator of immunoglobulin Fc receptors.¹⁹ Our study shows that Fc ϵ RI is upregulated by IL-4 and GM-CSF. We also found a lower expression of this IgE receptor on the MoDCs of atopic patients. Because all cultures took place under standard conditions, the intrinsic properties of monocytes of atopic and normal control subjects have to underlie this difference. Small differences were found for the expression of IgG receptors CD16 and CD64 and the IgA receptor CD89. These results were confirmed in a parallel study in which we found a similar decreased

expression of CD16, CD64, and CD89 on MoDCs from atopic nonasthmatic subjects (data not shown). We therefore conclude that the make-up of the Fc receptor phenotype of MoDC is altered in atopic disease. For some of these Fc receptors it has been shown that they are involved in antigen presentation.²⁰⁻²³ Therefore shown differences might result in an enhanced antigen uptake, processing, and presentation of allergen to antigen-specific T cells in vivo.

Although the role of leukocytes (e.g., T cells and eosinophils) in allergic disease has been studied thoroughly, little attention has been paid so far to the role of the DC. This lack of attention is probably due to practical inconvenience, such as the low frequency of this cell in all tissues and the lack of specific markers. Some studies suggest a regulatory role for the DC in the pathogenesis of atopic disease.^{24, 25} We have shown that in atopic subjects circulating DC precursors are potentially better antigen-presenting cells than in normal control subjects. The mechanisms involved are presently under study, but our data have clearly shown that in atopic subjects a discrepancy already exists at the blood-cell level. It is attractive to hypothesize that the increased accessory potency of the DCs of atopic subjects disturbs the delicate balance between inhibition and stimulation of T lymphocytes. Together with local factors and a different antigen uptake, this might lead to the impaired T-cell activation that forms the base of atopic inflammation.

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