

Functional plasticity of human respiratory tract dendritic cells: GM-CSF enhances T_H2 development

Alexander Faith, PhD,^a Joanne McDonald, MSc,^a Emma Peek, MSc,^a David Richards, MSc,^a Jason Caulfield, MSc,^a Elfy Chevetton, MD,^b David Roberts, MD,^b Tak Lee, MD, DSc,^a Christopher Corrigan, MD, PhD,^a and Catherine Hawrylowicz, PhD^a *London, United Kingdom*

Background: Dendritic cells within the human respiratory mucosa (RTDCs) are proposed to initiate immune responses to foreign antigens. Their capacity to polarize T-cell responses, however, has not been investigated.

Objective: To compare RTDCs with peripheral blood dendritic cells (PBDCs) with regard to phenotype, cytokine production, capacity to polarize T-cell responses, and effects of exposure to the pleiotropic cytokine, GM-CSF.

Methods: CD1a⁺ RTDCs and CD1c⁺ PBDCs were purified from nasal turbinates of patients with nonatopic rhinitis and peripheral blood of healthy individuals, respectively. In some experiments, matched CD1c⁺ RTDCs and PBDCs from patients with rhinitis were compared. The phenotype of DC was examined by flow cytometry and cytokine production by cytometric bead array. DCs were cocultured with allogeneic naive CD4⁺ T cells, and cytokine production was determined by immunophenotyping, cytometric bead array, and ELISA.

Results: Both RTDCs and PBDCs exhibited an immature phenotype, but RTDCs expressed lower levels of MHC class II antigen. Cross-linking of CD40 on PBDCs, but not RTDCs, induced production of IL-12p70. In mixed lymphocyte cultures, RTDCs induced a T_H1/T_H2 profile, whereas PBDCs induced a T_H1 profile. Exposure of RTDCs to GM-CSF induced a T_H2 pattern of response in the mixed lymphocyte cultures. In contrast, exposure of PBDCs to GM-CSF promoted a T_H1 response.

Conclusion: This report emphasizes the importance of studying tissue-derived primary DCs, demonstrates functional plasticity of RTDCs, and implicates GM-CSF in amplifying the potential

of RTDCs to initiate T_H2 responses in the airways. (*J Allergy Clin Immunol* 2005;116:1136-43.)

Key words: Human respiratory tract, dendritic cells, GM-CSF, T_H1/T_H2

Immune responses are under strict control in the respiratory tract of normal healthy individuals to maintain the integrity of the airway epithelium.¹ Disturbance of this homeostasis within the respiratory tract is a primary cause of inflammatory disorders such as rhinitis and asthma. Dendritic cells (DCs), the most potent antigen-presenting cell population, are uniquely capable of initiating a primary immune response.^{2,3} Furthermore, depletion of DCs has been shown to abrogate airway inflammation in sensitized mice responding to local challenge.⁴ However, the potential of DCs within the human airways to initiate and sustain inflammation remains unclear.

Current evidence indicates that DCs are highly heterogeneous, but 2 major populations of human DCs—myeloid-like, which include Langerhans cells and interstitial DC, and plasmacytoid-like, which are mainly located in the blood and secondary lymphoid organs—have been identified.⁵ Peripheral blood-derived myeloid and plasmacytoid DCs were originally reported to induce T_H1 and T_H2 responses,⁶ respectively. However, it has become increasingly evident that DCs, rather than being inherently committed to differentiation of CD4⁺ T cells into T_H1 and T_H2 cells, retain flexibility in their responses.^{3,4,7}

Studies over the past decade have identified myeloid DCs in the human respiratory mucosa.⁸⁻¹¹ CD1a was originally classified as a Langerhans cell marker in the epidermis but has been widely used to identify DCs in the respiratory tract.^{9,12-14} More recently, the major DC population within the nasal mucosa of normal, healthy individuals has been identified as bearing the myeloid DC marker, CD1c.¹¹ In contrast, few plasmacytoid DCs have been detected in the normal respiratory tract, although nasal allergen challenge induced recruitment of plasmacytoid DCs to the respiratory mucosa of sensitized individuals.¹⁵

The tissue environment, in particular the level of the T_H1-promoting cytokine, IL-12, is an important determinant of the pattern of T-cell responses.^{3,16} The pleiotropic cytokine, GM-CSF, has been demonstrated to abrogate

From ^athe Department of Asthma, Allergy and Respiratory Science, and ^bthe Ear, Nose and Throat Department, Guy's, King's and St Thomas' School of Medicine.

Disclosure of potential conflict of interest: Dr Corrigan has a consultant agreement with Altana Pharma UK and receives research support from unrestricted research grants from GlaxoSmithKline UK. The other authors reported no conflicts of interest.

Supported by a project grant from the British Lung Foundation. Dr Faith was supported by a fellowship from King's College, London. Dr Lee is an Asthma UK Professor, and his work is supported by a Medical Research Council program grant.

Received for publication December 1, 2004; revised July 13, 2005; accepted for publication August 1, 2005.

Available online October 3, 2005.

Reprint requests: Alexander Faith, PhD, Department of Asthma, Allergy and Respiratory Science, 5th Floor Thomas Guy House, Guy's Hospital, London, SE1 9RT, United Kingdom. E-mail: alex.faith@kcl.ac.uk.

0091-6749/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2005.08.002

Abbreviations used

DC:	Dendritic cell
MLR:	Mixed lymphocyte reaction
PBDC:	Peripheral blood dendritic cell
RTDC:	Respiratory tract dendritic cell
TLR4:	Toll-like receptor 4
UK:	United Kingdom

production of IL-12 by murine Langerhans cells¹⁷ and to be significantly elevated in the airways of atopic individuals with asthma compared with nonatopic individuals.¹⁸ Furthermore, animal experiments have shown GM-CSF to facilitate T_H2 responses.^{16,19,20} However, it is not clear whether GM-CSF can promote T_H2 responses by direct effects on human DCs.

The limited published studies of purified human respiratory tract DCs (RTDCs) have relied on investigations of bronchoalveolar lavage–derived DCs, which are present in low frequency,⁹ or lung tissue from carcinoma patients containing a high proportion of DCs from areas distal to the conducting airways.¹⁰ We have taken advantage of a new surgical technique designed to alleviate rhinitis, degloving of the inferior nasal turbinate,²¹ to isolate and purify sufficient numbers of RTDCs from the conducting airways to perform functional studies. We have compared the ability of RTDCs with that of peripheral blood–derived myeloid DCs to stimulate cytokine production by naive CD4⁺ T cells and addressed the hypothesis that RTDCs induce differentiation of T_H2 cells and that this process is enhanced by GM-CSF.

METHODS

Patients

Nasal tissue was obtained from 10 nonatopic patients (defined by negative skin prick test results for a range of 10 common aeroallergens) with chronic rhinitis undergoing inferior turbinectomy by the degloving technique. Patients age 18 to 65 were nonsmokers, had not received topical corticosteroids for at least 2 weeks before surgery, and had no other concurrent serious illness. Peripheral blood was obtained from nonatopic, age-matched and sex-matched healthy subjects. In further experiments, nasal tissue and blood were obtained concurrently from 2 patients with nonatopic rhinitis. The study was approved by Guy's Hospital Ethics Committee. All patients gave written informed consent before inclusion in the study.

mAbs

A panel of mAb to leukocyte surface markers conjugated to phycoerythrin or fluorescein isothiocyanate was obtained from BD Biosciences (Oxford, United Kingdom [UK]): CD1a, CD3, CD14, CD16, CD19, CD40, CD80, CD83, CD86, CD116, CD123, HLA-DR, and CCR6. mAb to Toll-like receptor 4 (TLR4) was obtained from SeroTec (Oxford, UK), and to CCR7 from R & D Systems (Abingdon, UK). Unconjugated anti-CD1c mAb was a generous gift of Dr S. Porcelli (Albert Einstein College of Medicine, Bronx, NY).

Isolation and purification of DCs

Tissue fragments were rinsed with saline to remove residual blood. Tissue was dissected, teased apart, and digested in HBSS with

endotoxin-free collagenase (2 mg/mL, Liberase C1; Roche Diagnostics, Lewes, UK) for 1 hour at 37°C. The digest was then centrifuged at 200g and cultured for 4 hours in RPMI1640 containing 10% FCS, 2 mmol/L L-glutamine and gentamycin (250 µg/mL), designated complete medium. The digested fragments were filtered through a 100-µm cell strainer (BD Biosciences) and a single cell suspension obtained. Cells were then positively selected on the basis of CD1a or CD1c expression on a magnetic column (according to the manufacturer's instructions; Miltenyi Biotec, Bisley, UK). The resulting DC population was 85% to 92% CD1a⁺ or CD1c⁺ containing <5% CD3⁺ cells and <3% CD14⁺ and CD19⁺ cells. For purification of DCs from peripheral blood, PBMCs were isolated by density gradient centrifugation (Lymphoprep; Axis-Shield, Oslo, Norway) and enzymatically digested as described to ensure similar treatment for tissue and blood-derived DCs. Peripheral blood DCs (PBDCs) were positively selected by using antibody-coated CD1c beads (Miltenyi Biotec). The resulting population was 90% to 95% HLA-DR⁺CD11c⁺ containing <4% CD14⁺ cells and <2% CD20⁺ and CD3⁺ cells.

Fluorescence-activated cell sorting analysis of purified DC populations

Samples were analyzed by using 2-color staining with conjugated mAbs. Isotype controls included the appropriate fluorochrome conjugated or unconjugated mouse IgG₁ or IgG_{2a} (Sigma, Poole, UK) and were concentration matched for each antibody used. Unconjugated anti-CD1c antibody was stained with a F(ab')₂ goat antimouse IgG-phycoerythrin secondary reagent (R & D Systems). The cells were washed, fixed, and then analyzed by flow cytometry using FACScan and CellQuest software (BD Biosciences). For analysis of specific epitope expression by DCs, cells were gated for large forward scatter cells and 5 to 10,000 events collected. All steps were performed at 4°C to prevent receptor internalization.

Cytokine production by purified DCs

Respiratory tract dendritic cells and PBDCs were seeded at 5×10^4 /well (96-well flat bottom) in complete medium (100 µL). DCs were stimulated with soluble, trimeric recombinant CD40L (1 µg/mL; Alexis, Nottingham, UK). Time course experiments demonstrated optimal production of cytokines after 48 hours (data not shown).

Cytometric bead array and IL-13 ELISA

The cytokine groups, IL-6, IL-8, IL-10, IL-12, TNF-α or IL-2, IL-4, IL-5, IL-10, and IFN-γ were measured simultaneously by cytometric bead array using the Human Inflammation Kit or T_H1/T_H2 kit, respectively (according to the manufacturer's instructions; BD Biosciences). The lower detection limit of the assay for each cytokine was 5 pg/mL. The IL-13 ELISA was performed by using a matched antibody pair (BD Biosciences), and the lower limit of detection was 100 pg/mL.

Coculture of RTDCs and PBDCs with CD4⁺CD45RA⁺ T cells

Peripheral blood was obtained from healthy, nonatopic individuals. CD4⁺ cells were purified from PBMCs by positive selection (Detachabead; Dynal, Oslo, Norway), and then CD45RA⁺ cells were obtained by negative selection using a CD45RO⁺ mAb (Pharmingen) and pan mouse immunoglobulin magnetic beads (Dynal). The resulting cell population was >98% CD4⁺CD45RA⁺. Purified DCs (1×10^6 /mL), precultured in medium alone or for 16 hours with GM-CSF (50 ng/mL), were washed twice and cocultured at a DC:T ratio of 1:10 with allogeneic CD4⁺CD45RA⁺ T cells (1×10^5 ; round bottom 96-well plate) or 1×10^6 (24-well plates) and

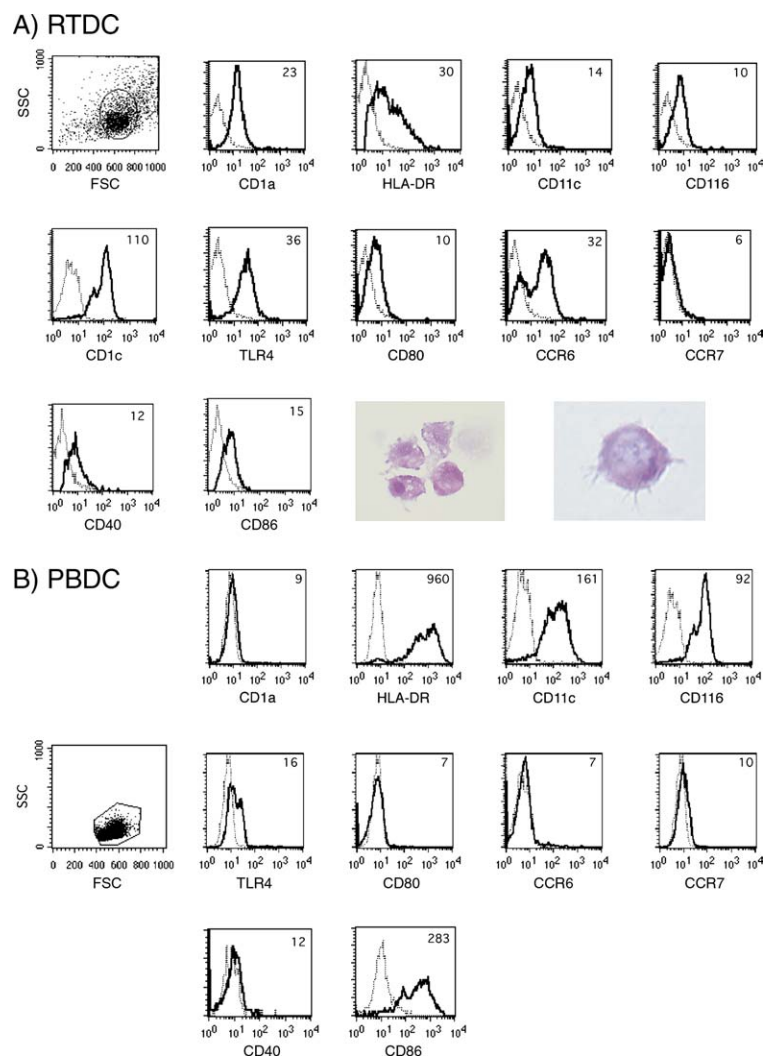


FIG 1. Surface phenotype of purified RTDCs (**A**) and PBDCs (**B**) as determined by flow cytometry. *Thin lines* represent staining with isotype-matched control antibody. *Numbers* represent mean fluorescence intensity. Morphology of RTDCs by hematoxylin and eosin staining is shown. One experiment representative of 3 is shown. *SSC*, Side scatter; *FSC*, forward scatter.

supernatants obtained after 6 days. T cells were then harvested and restimulated for 48 hours with previously optimized concentrations of mAb to anti-CD3 (1 μ g/mL; OKT3, purified in-house) and mAb to anti-CD28 (2 μ g/mL; CLB-CD28; Netherlands Red Cross, Amsterdam, The Netherlands).

Intracellular cytokine production

All reagents for intracellular staining were obtained from BD Biosciences. Phorbol 12-myristate 13-acetate (10 ng/mL; Sigma) and ionomycin (250 ng/mL; Calbiochem, Beeston, UK) were added to cultures for the final 4 hours and monensin for the final 2 hours to allow accumulation of cytokines within the cell. Cytofix/Cytoperm Plus (BD Biosciences) was then added and the cells permeabilized and double-stained with specific mAb to IL-13 conjugated to phycoerythrin and IFN- γ conjugated to fluorescein isothiocyanate. Appropriate fluorochrome conjugated isotype controls were included. Live T cells were gated on the basis of forward scatter characteristics and nonincorporation of the dye, 7-amino actinomycin D.

Statistics

The 2-tailed *t* test was used to compare cytokine production between groups.

RESULTS

Phenotype of myeloid DCs from the respiratory tract and peripheral blood

Nasal turbinates from 10 nonatopic patients with chronic rhinitis were enzymically digested and the composition of released cells determined. The myeloid DC marker, CD1a, was borne by 14 ($\pm$ 12) % (mean $\pm$ [1 SD]) of released cells. In contrast, only 3 ($\pm$ 2) % of cells expressed CD123, a marker of plasmacytoid DCs. Because CD1a⁺ DCs represented a significant proportion of cells released from turbinates and may possess critical functions in the airways,¹⁴ RTDCs were purified on the

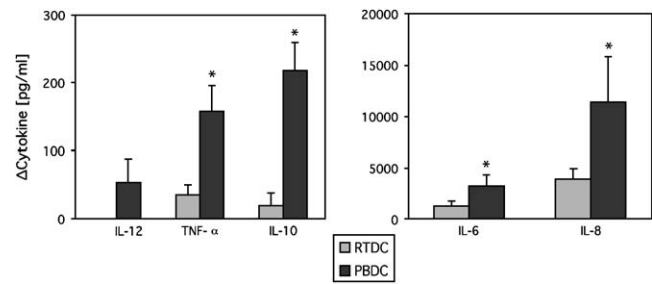


FIG 2. Cytokine production by RTDCs and PBDCs (5×10^4 /well) stimulated with soluble, trimeric CD40L. Supernatants were harvested after 48 hours and each cytokine determined. The results are shown as the means + 1 SDs of cytokine concentrations ($n = 5$). Background production of each cytokine was subtracted from the experimental value. *Significantly increased cytokine production ($P < .05$).

TABLE I. Cytokine production in the primary MLR

n = 5	IL-2	IL-4	IL-5	IL-10	IL-13	IFN-γ
RTDC (unstimulated)	72 (15)*	ND	82 (28)	20 (11)	147 (77)	83 (34)
PBDC (unstimulated)	135 (32)	ND	65 (45)	25 (8)	106 (45)	411 (94)†
RTDC (GM-CSF)	65 (22)	ND	124 (64)	35 (19)	184 (82)	65 (37)
PBDC (GM-CSF)	145 (46)	ND	79 (20)	40 (22)	120 (43)	345 (122)†

ND, Not detectable.
*Mean concentration, pg/mL (1 SD).
†Significant difference (RTDC vs PBDC; $P < .01$).

basis of CD1a expression and their phenotype and morphology investigated. To investigate distinct features of RTDCs, the cells were compared with myeloid DCs purified from peripheral blood of healthy individuals by a standard method using anti-CD1c coated magnetic beads, because CD1a is not expressed by PBDCs.²² Respiratory tract DCs lacked expression of markers of T, B, and natural killer cells and monocytes/macrophages (data not shown). RTDCs possessed an irregular outline and oval nucleus with many cells characterized by numerous fine dendrites (Fig 1, A). RTDCs expressed low levels of the MHC class II molecule, HLA-DR (Fig 1, A). Approximately 50% of RTDCs expressed CD116 (granulocyte-monocyte colony stimulating factor receptor [GM-CSFR]) and the myeloid DC marker, CD11c. RTDCs lacked expression of the maturation marker, CCR7, but the majority of cells bore the mucosal DC marker, CCR6. RTDCs strongly expressed the Toll-like receptor 4, TLR4, a pathogen pattern receptor binding to LPS, and also coexpressed CD1a and CD1c, confirming a previous report.¹¹ PBDCs expressed 30-fold higher levels of HLA-DR compared with RTDCs (Fig 1, B). CD11c and CD116 were also more strongly expressed on PBDCs. In contrast, PBDCs did not express CD1a, CCR6, or CCR7 and expressed less TLR4 compared with RTDCs. The costimulatory molecules CD80, CD86, and CD40 were expressed weakly by RTDCs. In contrast, PBDCs expressed high levels of CD86, but CD40 and CD80 were barely detectable. Exposure of RTDCs to GM-CSF resulted in 3-fold and 4-fold increases of CD80 and CD86 expression, respectively (data not shown). RTDCs, therefore, exhibited features of immature DCs but

were clearly distinct from PBDCs in terms of receptor expression.

Cytokine production by RTDCs and PBDCs

Production of proinflammatory cytokines by RTDCs and PBDCs in response to CD40L, a surrogate for T-cell interaction,⁷ was determined. RTDCs did not secrete IL-12p70, the bioactive form of IL-12 (Fig 2). This contrasted with PBDCs, which responded to CD40L stimulation with production of IL-12p70 even though consistently low levels of expression of CD40 were observed. Both RTDCs and PBDCs upregulated TNF-α, IL-6, and IL-8, but to a significantly greater degree in PBDCs compared with RTDCs ($P < .05$). Production of the anti-inflammatory cytokine, IL-10, by RTDCs and PBDCs was detected and also significantly elevated in PBDCs compared with RTDCs ($P < .05$).

RTDCs and PBDCs induce distinct cytokine patterns in naive CD4⁺ T cells

The capacity of RTDCs and PBDCs to induce differentiation of naive T cells was investigated in the mixed lymphocyte reaction (MLR). To determine optimal ratios of RTDCs or PBDCs and T cells, allogeneic, naive CD4⁺ T cells (1×10^5) were cultured with graded numbers (5×10^2 to 2×10^4) of DCs, and IL-2 production was measured after 6 days. An optimal T:RTDC/PBDC ratio of 10:1 was determined (data not shown) and then used in all experiments. RTDCs induced low levels of both T_H1 and T_H2 cytokines (Table I), whereas PBDCs induced significantly higher levels of IFN-γ than RTDCs. T_H1 and T_H2 cytokine production were then determined after

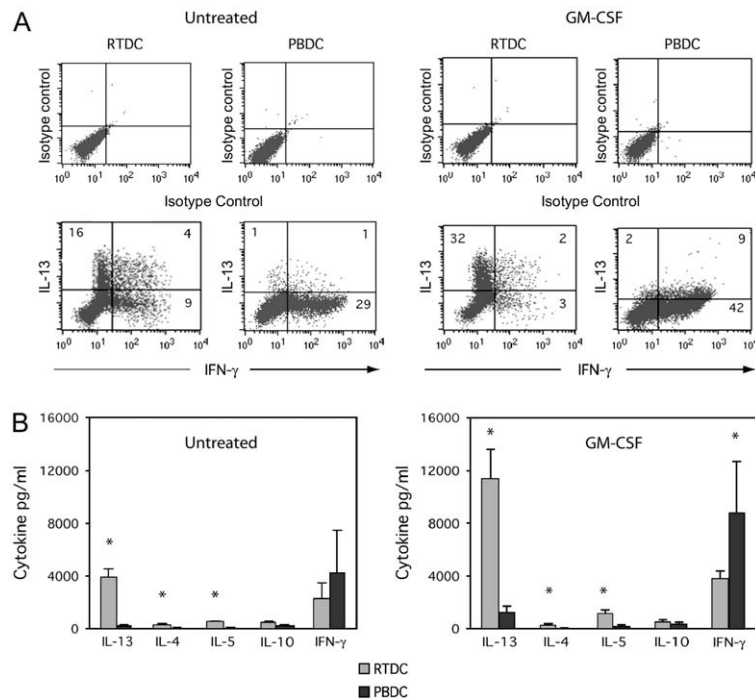


FIG 3. Effect of RTDCs and PBDCs on cytokine production by T cells. RTDCs or PBDCs were cultured with naive, allogeneic CD4⁺ T cells for 6 days, and T cells were then restimulated with mAbs to CD3/CD28. **A**, Numbers in each quadrant show the percentage of T cells immunophenotyped for IL-13 or IFN-γ. **B**, Cumulative data are expressed as means + 1 SDs cytokine concentrations (n = 5). **P* < .01, RTDC vs PBDC.

secondary stimulation by anti-CD3 and anti-CD28 of T cells harvested from the primary MLR. RTDCs induced a mixed T_H1 and T_H2 pattern of response detected by immunophenotyping of intracellular IL-13 and IFN-γ after stimulation with phorbol 12-myristate 13-acetate/ionomycin (Fig 3, A). This was confirmed by measurement of soluble cytokines secreted by T cells after anti-CD3/28 stimulation (Fig 3, B). High levels of IL-13 and IFN-γ, moderate amounts of IL-5, and low concentrations of IL-4 were detected. In contrast, PBDCs induced a T_H1 pattern in responding T cells. Production of IL-4, IL-5, and IL-13 induced by RTDCs was significantly elevated (*P* < .01) compared with PBDCs. Production of IL-10 (180–480 ng/mL) was comparably induced by tissue and blood DCs.

GM-CSF promotes T_H2 development by RTDCs

The effect of GM-CSF on polarization of T-cell responses was investigated. Previous exposure of RTDCs to GM-CSF did not affect T_H2 cytokine production in the primary MLR (Table I), but GM-CSF-treated PBDCs induced significantly greater IFN-γ production compared with similarly treated RTDC. After secondary stimulation, strong T-cell production of IL-13 and moderate levels of IL-5 and IFN-γ were induced (Fig 3, A and B). In contrast, previous exposure of PBDCs to GM-CSF induced strong T_H1 responses. GM-CSF-pretreated RTDCs, compared with PBDCs, induced significantly more IL-4, IL-5, and

IL-13 (*P* < .01) but less IFN-γ production (*P* < .05) in naive T cells after secondary stimulation. The effect of exposure of RTDCs and PBDCs to the prototype T_H1-inducing stimulus, LPS/IFN-γ, was also investigated. Exposure of both RTDCs and PBDCs to LPS/IFN-γ enhanced the T_H1 response (data not shown). Thus, the pattern of T_H2 cytokine production induced by RTDCs in naive T cells was distinct from that induced by PBDCs and could be enhanced by GM-CSF.

Phenotype of matched CD1c⁺ RTDCs and PBDCs

It was possible that the effects of RTDCs and PBDCs were attributable to the selection of distinct populations of cells from different individuals rather than their location. CD1c⁺ RTDCs and PBDCs were, therefore, purified from matched turbinate and peripheral blood of patients with rhinitis. The myeloid DC marker, CD1c, was borne by 6% to 8% of RTDCs released from nasal turbinates. RTDCs expressed low levels of costimulatory molecules CD80 and CD86 (Fig 4, A). Forty-five percent of CD1c⁺ RTDCs expressed CD1a. In contrast, PBDCs expressed high levels of CD86 but lacked expression of CD80 and CD1a. RTDCs expressed 10-fold lower levels of HLA-DR compared with PBDCs. Moreover, RTDCs, in contrast with PBDCs, failed to secrete IL-12 in response to CD40 ligation (data not shown). Thus, CD1c⁺ and CD1a⁺ RTDCs expressed a similar phenotype.

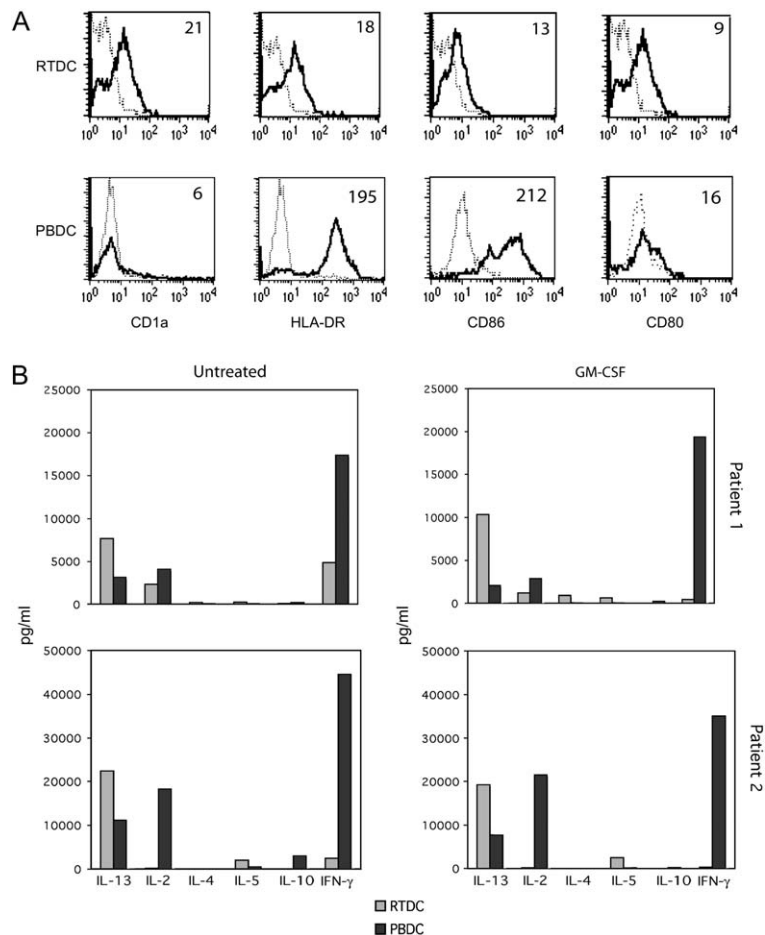


FIG 4. Surface phenotype of and cytokine production induced by matched CD1c⁺ RTDCs and PBDCs. **A**, *Thin lines* represent staining with isotype-matched control antibody. *Numbers* represent mean fluorescence intensity. **B**, RTDCs and PBDCs from 2 patients with rhinitis were untreated or pretreated with GM-CSF and cocultured with T cells as in Fig 3. Data represent cytokine production by T cells.

Distinct patterns of cytokine production induced by matched CD1c⁺ RTDCs and PBDCs in naive CD4⁺ T cells

T-cell cytokine production in the MLR was determined in response to matched RTDCs and PBDCs from 2 patients with rhinitis (Fig 4, B). Untreated RTDCs induced a mixed T_H1 and T_H2 cytokine profile in the MLR, although IFN- γ production induced by RTDCs from patient 2 was low (2442 pg/mL). RTDCs induced strong levels of production of IL-13. Thus, the pattern of cytokine production induced by RTDCs isolated on the basis of either CD1a or CD1c expression was similar. Exposure of RTDCs from both patients to GM-CSF ablated production of IFN- γ . GM-CSF-treated RTDCs from patient 1 enhanced T_H2 cytokine production, but there was little effect of GM-CSF-treated RTDCs from patient 2 on T_H2 cytokine production. In contrast, PBDCs induced a strong production of IFN- γ both before and after exposure to GM-CSF. Exposure of PBDCs from these patients to GM-CSF had little effect on T_H2 cytokine production.

DISCUSSION

The current study is one of few addressing the phenotype and functional properties of DCs isolated directly from the human respiratory tract. Our results highlight important differences between RTDCs and PBDCs in terms of their surface phenotype, cytokine expression, functional plasticity, and ability to polarize responses of naive T cells. Although previous studies have reported that RTDCs induced strong proliferative responses in allogeneic, unfractionated T cells,^{10,12} none has addressed the issue of T-cell differentiation. Our data suggest that RTDCs from individuals susceptible to airway inflammation have the capacity to provide the sustained activation required for differentiation of effector T cells. Murine models of sensitization in the airways support this contention. In such models, RTDCs throughout the conducting airways are rapidly susceptible to signals enhancing their competence to form complexes with and subsequently activate antigen-specific T cells.²³⁻²⁵

Functional studies demonstrated that RTDCs isolated on the basis of either CD1c or CD1a expression induced a mixed T_H1/T_H2 pattern of cytokine production in naive $CD4^+$ T cells, whereas PBDCs from patients and healthy individuals induced a much more strongly T_H1 pattern of response. The results obtained by using DCs from the same individuals indicated that differences in response induced by RTDCs and PBDCs reflected the origin of the DCs rather than the disease state of the patients. We suggest that there are 3 possible mechanisms contributing to these results. First, RTDCs expressed relatively low densities of MHC class II compared with PBDCs. This may allow low affinity interactions between RTDCs and T cells, which have been shown to favor T_H2 differentiation.^{3,26} Second, RTDCs did not express measurable amounts of IL-12, central to initiation of a dominant T_H1 response, after CD40 ligation. The reported stringent control of IL-12 production by Langerhans cells²⁷ as well as RTDCs may reflect the necessity to constrain T_H1 -mediated inflammatory reactions at mucosal sites. Third, RTDCs, in contrast with PBDCs, expressed CD80 directly *ex vivo*. We have observed that both CD80 and CD86 costimulation is essential for IL-5 but not IFN- γ production in primary allogeneic T-cell responses (data not shown). Human gut-derived mucosal DCs have also been shown recently to induce T_H2 responses.²⁸ The capacity to induce T_H2 responses may, therefore, be an inherent feature of human mucosal, as distinct from peripheral blood, immunity.

Myeloid RTDC populations purified on the basis of CD1c or CD1a expression induced differentiation of T_H2 cells. Peripheral tissue DCs are believed to derive from circulating monocyte-like precursors.²⁹ CD1a/CD1c⁺ RTDCs are confined to the airway epithelium, whereas CD1c⁺ RTDCs are located in both the epithelium and lamina propria.¹¹ Environmental stimuli encountered by a subset of CD1c⁺ RTDCs on migration to or location in the epithelium may induce upregulation of CD1a.³⁰ Both CD1a/CD1c⁺ and CD1c⁺ RTDCs, however, expressed low levels of MHC class II and costimulatory molecules, indicating a lack of maturation of RTDCs within the epithelium.

The proinflammatory cytokine GM-CSF enhanced the T_H2 response induced by RTDCs in the MLR. The result was particularly marked in experiments with CD1c⁺ RTDCs, in which production of IFN- γ was ablated. In contrast, exposure of PBDCs to GM-CSF promoted T_H1 development in allogeneic T cells. One possible explanation for this may lie in the differential capacity of RTDCs and PBDCs to produce TNF- α in response to CD40 ligation. GM-CSF alone was unable to upregulate IL-12 production by murine DCs but required TNF- α as costimulus.³¹ Deficiency in production of this factor by RTDCs, therefore, may skew the response toward a T_H2 pattern. The results support the hypothesis that RTDCs exhibit a less mature or distinct state of differentiation.

The results from this study emphasize the critical importance of studying tissue-derived primary human

DCs. Although we were unable to perform comparative experiments on turbinates from normal, healthy individuals for ethical reasons, the results support the concept of functional plasticity of human RTDCs, a previously unaddressed issue. This plasticity offers a rationale for considering intervention strategies designed to target DCs at the lesional site in mucosal inflammatory diseases such as asthma or rhinitis.

We are grateful to Mrs Kheem Jones for performing skin prick tests on patients. We thank Drs Sun Ying and Qiu Meng for morphological analysis of RTDCs.

REFERENCES

- Holt PG, Stumbles PA. Regulation of immunologic homeostasis in peripheral tissues by dendritic cells: the respiratory tract as a paradigm. *J Allergy Clin Immunol* 2000;105:421-9.
- Boonstra A, Asseloin-Paturel C, Gilliet M, Crain C, Trinchieri G, Liu Y-J, et al. Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing Th helper 1 and 2 cell development: dependency on antigen dose and differential toll-like receptor ligation. *J Exp Med* 2003;197:101-9.
- Viera PL, de Jong EC, Wierenga EA, Kapsenberg ML, Kalinski P. Development of Th1-inducing capacity in myeloid dendritic cells requires environmental instruction. *J Immunol* 2000;164:4507-12.
- Lambrecht BN, Salamon B, Klatzmann D, Pauwels RA. Dendritic cells are required for the development of chronic eosinophilic airway inflammation in response to inhaled antigen in sensitised mice. *J Immunol* 1998;160:4090-7.
- Itoh T, Inaba M, Inaba K, Toki J, Sogo S, Iguchi T, et al. A CD1a/CD11c subset of human blood dendritic cells is a direct precursor of Langerhans cells. *J Immunol* 1999;163:1409-19.
- Rissoan MC, Soumelis V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R, et al. Reciprocal control of T helper cell and dendritic cell differentiation. *Science* 1999;283:1183-6.
- Cella M, Facchetti F, Lanzavecchia A, Colonna M. Plasmacytoid dendritic cells activated by influenza virus and CD40-ligand drive a potent Th1 polarisation. *Nat Immunol* 2001;5:919-23.
- Fokkens WJ, Vroom TM, Rjintjes E, Mulder P. HLA-DR expressing cells, presumably Langerhans cells, in nasal mucosa. *Allergy* 1989;44:167-72.
- van Haarst JMW, Hoogsteden HC, de Wit HJ, Verhoeven GT, Havenith CE, Drexhage HA. Dendritic cells and their precursors isolated from human bronchoalveolar lavage: immunocytologic and functional properties. *Am J Respir Cell Mol Biol* 1994;10:487-92.
- Cochand L, Isler P, Songeon F, Nicod LP. Human lung dendritic cells have an immature phenotype with efficient mannose receptors. *Am J Respir Cell Mol Biol* 1999;21:547-54.
- Jahnsen FL, Gran E, Haye R, Brandtzaeg P. Human nasal mucosa contains antigen-presenting cells of strikingly different functional phenotypes. *Am J Respir Cell Mol Biol* 2003;30:31-7.
- van Harst JM, Voerhoeven GT, de Wit HJ, Hoogsteden HC, Debets R, Drexhage HA. CD1a⁺ and CD1a⁻ accessory cells from human bronchoalveolar lavage differ in allostimulatory potential and cytokine production. *Am J Respir Cell Mol Biol* 1996;15:752-9.
- Tunon-de-Lara JM, Redington AE, Bradding P, Church MK, Hartley JA, Semper AE, et al. Dendritic cells in normal and asthmatic airways: expression of the α subunit of the high affinity immunoglobulin E receptor (Fc ϵ RI- α). *Clin Exp Allergy* 1996;26:648-55.
- Till SJ, Jacobson MR, O'Brien F, Durham SR, Kleinjan A, Fokkens WJ, et al. Recruitment of CD1a⁺ Langerhans cells to the nasal mucosa in seasonal allergic rhinitis and effects of topical corticosteroid therapy. *Allergy* 2001;56:126-31.
- Jahnsen FL, Lund-Johansen F, Dunne JF, Farkas L, Haye R, Brandtzaeg P. Experimentally induced recruitment of plasmacytoid (CD123^{high}) dendritic cells in human nasal allergy. *J Immunol* 2000;165:4062-8.

16. Pulendran B, Smith JL, Caspary G, Brasel K, Pettit D, Maraskovsky E, et al. Distinct dendritic cell subsets differentially regulate the class of immune response in vivo. *Proc Natl Acad Sci U S A* 1999;96:1036-41.
17. Tada Y, Asahina A, Nakamura K, Miyazono K, Tomura M, Fujiwara H, et al. Transforming growth factor-beta up-regulates CD40-engaged IL-12 production of mouse Langerhans cells. *Eur J Immunol* 2001;31:294-300.
18. Sousa AR, Poston RN, Lane SJ, Nakhosteen JA, Lee TH. Detection of GM-CSF in asthmatic bronchial epithelium and decrease by inhaled corticosteroids. *Am Rev Respir Dis* 1993;147:1557-61.
19. Vasu C, Dogan R-N, Holterman MJ, Prabhakar B. Selective induction of dendritic cells using granulocyte macrophage-colony stimulating factor, but not fms-like tyrosine kinase receptor 3-ligand, activates thyroglobulin-specific CD4+/CD25+ T cells and suppresses experimental autoimmune thyroiditis. *J Immunol* 2003;170:511-22.
20. Cates EC, Gajewska BU, Goncharova S, Alvarez D, Fattouh R, Coyle AJ, et al. Effect of GM-CSF on immune, inflammatory, and clinical responses to ragweed in a novel model of mucosal sensitisation. *J Allergy Clin Immunol* 2003;111:1076-86.
21. Chevreton EB, Hopkins C, Black IM, Tierney P, Smeeton NC. Degloving of the inferior turbinates: pilot study to assess the effectiveness of a new technique in turbinate reduction. *J Laryngol Otol* 2003;117:866-70.
22. Schnurr M, Toy T, Stoitzner P, Cameron P, Shin A, Beecroft T, et al. ATP gradients inhibit the migratory capacity of specific human dendritic cell types: implications for P2Y11 receptor signaling. *Blood* 2003;102:613-20.
23. Julia V, Hessel EM, Malherbe L, Glaichenhaus N, O'Garra A, Coffman RL. A restricted subset of dendritic cells captures airborne antigens and remains able to activate specific T cells long after antigen exposure. *Immunity* 2002;16:271-83.
24. Huh JC, Strickland DH, Jahnsen FL, Turner DJ, Thomas JA, Napoli S, et al. Bidirectional interactions between antigen-bearing respiratory tract dendritic cells (DCs) and T cells precede the late phase reaction in experimental asthma. *J Exp Med* 2003;198:19-30.
25. Constant SL, Brogdon JL, Piggott DA, Herrick CA, Visintin IN, Ruddle NH, et al. Resident lung antigen-presenting cells have the capacity to promote Th2 T cell differentiation in situ. *J Clin Invest* 2002;110:1441-8.
26. Carballido J, Faith A, Carballido-Perig N, Blaser K. The intensity of T cell receptor engagement determines the cytokine pattern of human allergen-specific T helper cells. *Eur J Immunol* 1997;27:515-21.
27. Goddard S, Youster J, Morgan E, Adams DH. Interleukin-10 secretion differentiates dendritic cells from human liver and skin. *Am J Pathol* 2004;164:511-9.
28. Rimoldi M, Chieppa M, Salucci V, Avogadri F, Sonzogni A, Sampietro GM, et al. Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nat Immunol* 2005;6:507-14.
29. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* 2003;19:71-82.
30. Kohrgruber N, Halanek N, Groger M, Winter D, Rappersberger K, Schmitt-Egenolf M, et al. Survival, maturation and function of CD11c- and CD11c+ peripheral blood dendritic cells are differentially regulated by cytokines. *J Immunol* 1999;163:3250-9.
31. Stumbles PA, Thomas JA, Pimm CL, Lee PT, Venaille TJ, Proksch S, et al. Resting respiratory tract dendritic cells preferentially stimulate T helper cell type 2 (Th2) responses and require obligatory cytokine signals for induction of Th1 immunity. *J Exp Med* 1998;188:2019-31.