

Comparative analysis of nasal and oral mucosa dendritic cells

Background: Mucosal dendritic cells (DC) play a crucial role in tolerance induction as seen in mucosal immunotherapy of atopic diseases. Nevertheless little is known about the phenotypical differences of oral and nasal mucosal DC (nmDC). Recently, we could show that oral mucosal myeloid CD1a⁺ DC (omDC) differ from their skin counterparts especially by the expression of high affinity receptor for immunoglobulin E (IgE; FcεRI). However, expression pattern of FcεRI and phenotypical characteristics of CD1a⁺ nmDC have not been elucidated in detailed yet.

Methods: We performed detailed phenotypical comparison of nmDC and omDC of atopic and nonatopic individuals.

Results: As reported for omDC, FcεRI on nmDC of atopic donors was elevated and mostly occupied by IgE while FcεRI was present only in low amounts on nmDC of nonatopic donors. Nevertheless, the highest FcεRI expression has been observed on omDC. Furthermore, significant amounts of costimulatory molecules CD40, CD80 and CD86 could be detected on nmDC that expressed more CD80 compared with omDC. Moreover, nmDC displayed less major histocompatibility complex (MHC) class I and II molecules than omDC. In addition, nmDC expressed more C-type lectins CD205, CD206 as well as myeloid marker CD11b while omDC displayed increased expression of CD207 and lipopolysaccharide (LPS) receptor CD14.

Conclusion: Together these data imply that nmDC phenotypical differ from omDC which might result in diverse functional properties and might be of relevance for selecting routes for immunotherapy of atopic diseases. Moreover these data provide a basis for further studies investigating immunological mechanisms underlying mucosal immunotherapy.

**J.-P. Allam¹, B. Niederhagen²,
M. Bücheler³, T. Appel², H. Betten¹,
T. Bieber¹, S. Bergé², N. Novak¹**

Departments of ¹Dermatology ²Oral and Maxillofacial Surgery and ³Oto-Rhino-Laryngology, Head and Neck Surgery, University of Bonn, Bonn, Germany

Key words: dendritic cells; high affinity receptor for immunoglobulin E; immunotherapy; mucosa; nasal; oral.

Natalija Novak MD
Department of Dermatology
Sigmund-Freud-Str. 25
53105 Bonn
Germany

Accepted for publication 19 July 2005

Local immunotherapy represents a promising treatment of atopic diseases just as allergic rhinitis and allergic asthma. So far, oral and nasal immunotherapy has been shown to be efficient and most likely local dendritic cells (DC) as antigen-presenting cells (APC) are involved in the immunological processes leading to a reduction of allergic symptoms after immunotherapy. It has been proposed that successful immunotherapy is partly mediated by a shift from an allergy-tended Th2 towards a Th1 immune response whereas DC are critically involved in this process (1). The DC represent outposts of the immune system, which are located at the border zones of our organisms to the environment, such as the oral, or the nasal mucosa (2). So far two different subsets of DC involved in mucosal immunity have been described – CD1a⁺ myeloid and CD123⁺ plasmacytoid DCs (pDCs).

During local immunotherapy the mucosal tissue is exposed to applied allergens such as house dust mite allergens, birch or grass pollen allergens and most likely immune reactions differ within nasal and oral site of allergen application (3). DC – as APC – are able to trap these allergens either via endocytosis receptors like C-type lectins or via allergen-specific surface-bound immunoglobulin E (IgE) and its receptors, internalize them and migrate to the regional lymph nodes to present the processed allergens to T cells (4, 5). It has been shown lately that both pathways are involved in allergen uptake *in vitro* (6, 7). Moreover the micro-environment, which is dictated by microbial flora in the nasal and oral mucosa, might also have an impact on local DC (8).

Recently, we could demonstrate that oral mucosal DC (omDC) constitutively express FcεRI and it has been shown that surface expression of FcεRI on DC is deeply dependent on cellular and soluble factors in the micro-environment, especially the presence of IgE molecules (9–11). It is known that IgE is present in enhanced

Abbreviations: rFI, relative fluorescence index; rSI, relative stimulation index; FcεRI, high affinity receptor for IgE.

amounts in the nasal mucosa of patients with allergic rhinitis and might therefore influence the surface expression of FcεRI–IgE complexes and the phenotype DC (12). Most importantly, current therapeutic strategies of allergic asthma and allergic rhinitis such as the application of anti-IgE antibodies target IgE–FcεRI complexes on effector cells and DC (13).

It is obvious that understanding the factors which influence the FcεRI/IgE surface expression and the identification of differences in the expression pattern of these structures in allergic diseases is indispensable for the development of effective therapeutic strategies of the future.

Moreover, as local immunotherapy represents a promising sufficient procedure in the cure of atopic diseases detailed characterization of local DC within the tissue of allergen application might contribute to the decision on which route is most effective. Furthermore, recent efforts in improving local immunotherapy tend to modify the formulation by adding adjuvants to improve therapeutic efficiency (14) so that detailed characterization of local DC types and their receptors might have great impact on the selection of potential adjuvants.

Therefore in the present study, we analysed the expression of IgE receptors and the constitutive IgE-binding of myeloid DC (mDC) isolated from nasal mucosa (nmDC) in comparison with omDC of atopic and nonatopic patients. Furthermore, we investigated their phenotypical characteristics in respect to lineage-specific markers contributing to antigen uptake.

Material and methods

Reagents

Phycoerythrin (PE)-labelled T6RD1 (IgG1; Coulter, Krefeld, Germany) recognizes CD1a and PE-labelled 9F5 (IgG1; Becton Dickinson, Mountain View, CA, USA) CD123. The high affinity receptor for IgE, FcεRI, was detected either by monoclonal antibody (mAb) 22E7 (IgG1; generous gift of Dr J. Kochan, Hoffmann La Roche Co., Nutley, NJ, USA), which is directed against the α-chain of FcεRI but does not interfere with the binding site for IgE or mAb 15A5 (IgG1; generous gift of Dr J. Kochan) which interferes with the binding site for IgE; mAb W6/32 against the major histocompatibility complex (MHC) class I-producing hybridoma cell line was kindly provided by Dr N. Koch (Institute of zoophysiology, University of Bonn, Germany), mAb IOT2b (IgG1; Immunotech, Marseille, France) reacts with human leucocyte antigen (HLA)-DR; E124.2.8 (IgG1; Immunotech) reacts with the Dε2 constant region of human IgE; mAb BO7.4/B7.5 (IgG1; Becton Dickinson) reacts with CD80; mAb B70/B7-2 (IgG1; BD Pharmingen, San Diego, CA, USA) is directed against CD86; mAb 5C3 is directed against CD40 (IgG1; BD Pharmingen). The mAb M5E2 (IgG2a) against CD14, mAb BEAR-1 (IgG1) detecting CD11b, mAb DCN46 (IgG2b) against DC-SIGN/CD209, mAb 19 (IgG1) detecting CD206 and Langerin/CD207 was from Immunotech. The mAb MG38 detecting CD205 was purchased from eBioscience (San Diego, CA, USA). MOPC-141 (IgG2b), MOPC-21 (IgG1), UPC10 (IgG2a; Sigma, Deisenhofen, Germany) and IgG1RD1 (Beckman-

Coulter, Krefeld, Germany) were used as appropriate isotype controls. Fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse (GaM/FITC) antibody was from Jackson Laboratories (West Grove, PA, USA). Normal mouse serum for blocking purposes was obtained from Dianova (Hamburg, Germany) and 7-amino-actinomycin-D (7-AAD) was from Sigma. Total serum IgE levels were determined by the immulite technique (DPC Biermann GmbH, Bad Nauheim, Germany).

Nasal and oral mucosa specimen

Nasal mucosa from hyperplastic inferior turbinates was obtained from patients (age 36 ± 15 years, $n = 31$) undergoing routine surgery procedures at the Department of Oto-Rhino-Laryngology of the University of Bonn. Specimen of oral mucosa from the vestibular region was obtained from patients undergoing intraoral surgery for extraction of the third molar or revision of mandibular fracture at the Department of Oral and Maxillofacial Surgery (age 33 ± 14 years, $n = 30$). All specimens were obtained after informed consent from patients according to the approval of the local ethics committee. In some patients serum samples were drawn additionally and processed for functional studies as described below. Atopics were defined by IgE serum levels > 100 kU/l with a history of atopic diseases and sensitization to common aeroallergens confirmed by skin prick test or allergen-specific IgE. Nonatopics were defined by IgE serum levels lower 100 kU/l without a history of atopic diseases lacking sensitization to common aeroallergens. Patients undergoing antihistamine, leukotrienes, β-mimetics or glucocorticoid treatment or suffering from a tumour have been excluded from the study.

Preparation of cell suspensions

Crude epithelial cell suspensions were prepared by trypsinization in a 0.5% trypsin buffer without Ca^{2+} for 2 h at 37°C as described elsewhere (15). Cell number obtained varied between $8\text{--}20 \times 10^6$ cells for nasal mucosa and $1\text{--}4 \times 10^6$ cells for oral mucosa.

Immunolabelling of cell suspensions

An indirect triple-staining for a number of $0.5\text{--}2 \times 10^5$ unfixed, vital cells was performed as described elsewhere (15). Finally, the cells were washed twice and analysed by flow cytometry.

Flow cytometric analysis

Cells were analysed on a FACS-Calibur (Becton Dickinson) as described in details elsewhere. For quantitative evaluation, dead cells were excluded by 7-AAD staining and the CD1a population was gated out manually. Trypsin sensitivity of investigated structures was excluded (data not shown). Data were determined using *LYSIS II* software and either expressed in percentage of positive cells or by relative fluorescence indices (rFI) of all structures were determined as follows.

$$\text{rFI} = [\text{MFI}(\text{receptor}) - \text{MFI}(\text{control})] / \text{MFI}(\text{control})$$

Statistical analysis

For statistical evaluation of significances, the Mann–Whitney *U*-test or Wilcoxon was performed. Correlations were calculated by Pearson's linear regression analysis. These tests were realized using the *SPSS 12.0* for Windows software. Results are shown as arithmetic mean $\pm$ SEM (**P* < 0.05; ***P* < 0.01; no indication, not significant).

Results

Distinctive composition of dendritic cell subsets within the nasal and oral mucosa

It has been reported that nasal mucosal tissue harbours CD123⁺ pDC as well as CD1a⁺ mDC whereas it could have been shown recently that especially CD123⁺ pDC are involved in tolerogenic mechanisms within the lung (16, 17). As nothing is known about the distribution of CD123⁺ pDCs within the oral mucosal tissue we compared

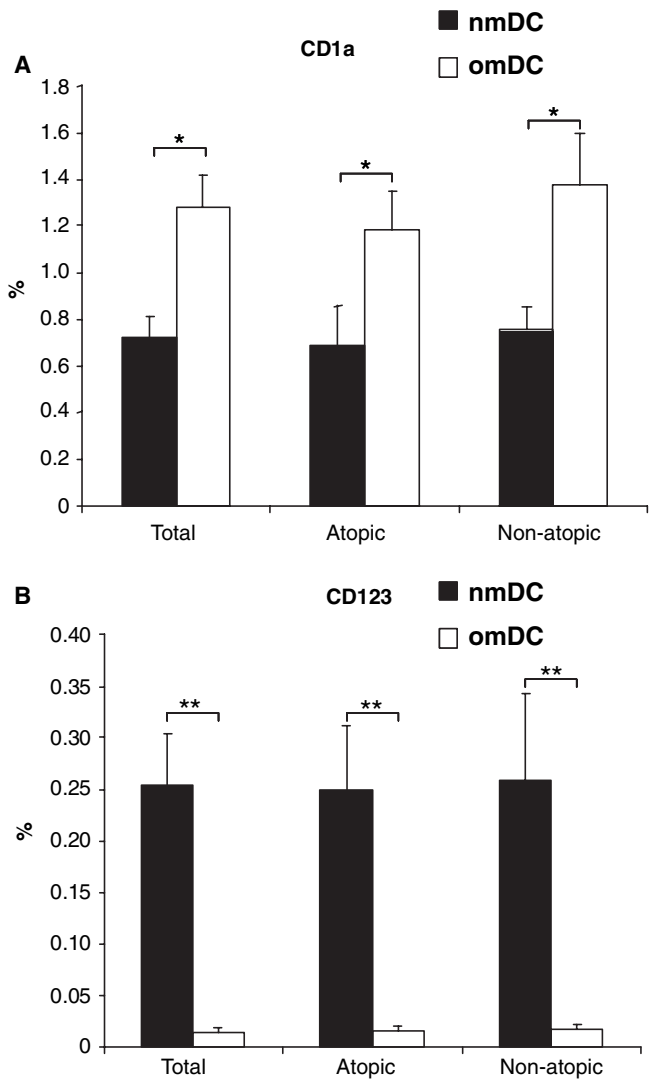


Figure 1. Distribution of plasmacytoid dendritic cell (pDC) and myeloid dendritic cell (mDC) within the nasal and oral mucosa single cells suspension was stained for flow cytometry. The mDC were detected by their CD1a and pDC by their CD123 expression and gated manually. Dead cells were excluded by 7-amino-actinomycin-D (7-AAD) dye. Significant higher percentage of CD1a⁺ myeloid DC were present within the oral mucosa while pDC were only found within the nasal mucosa (nmDC: *n* = 10, omDC: *n* = 10). No differences of pDC and mDC in normal nasal and oral mucosa could be seen between atopic and non-atopic donors. omDC, oral mucosal myeloid dendritic cells.

the presence of pDC in oral and nasal mucosal tissue of allergic and nonallergic patients. We could thereby detect mDC and pDC within the nasal mucosa of allergic and nonallergic patients while only mDC could be detected within the oral mucosa. Furthermore, the percentage of CD1a⁺ mDC was higher within the oral mucosa. No differences in DC percentage could be detected between atopic and nonatopic donors (Fig. 1A,B).

From this set of experiments we concluded that in contrast to nasal mucosa, oral mucosa only harbours mDC. So we proceeded to compare mDC within the nmDC to mDC of the omDC.

Differential expression of FcεRI on nasal mucosa CD1a⁺ DC of atopics and nonatopics

Recently, we could demonstrate that constitutive expression of FcεRI represents one unique feature of omDC. We could further show that its expression was even enhanced on omDC of atopic individuals so that we investigated

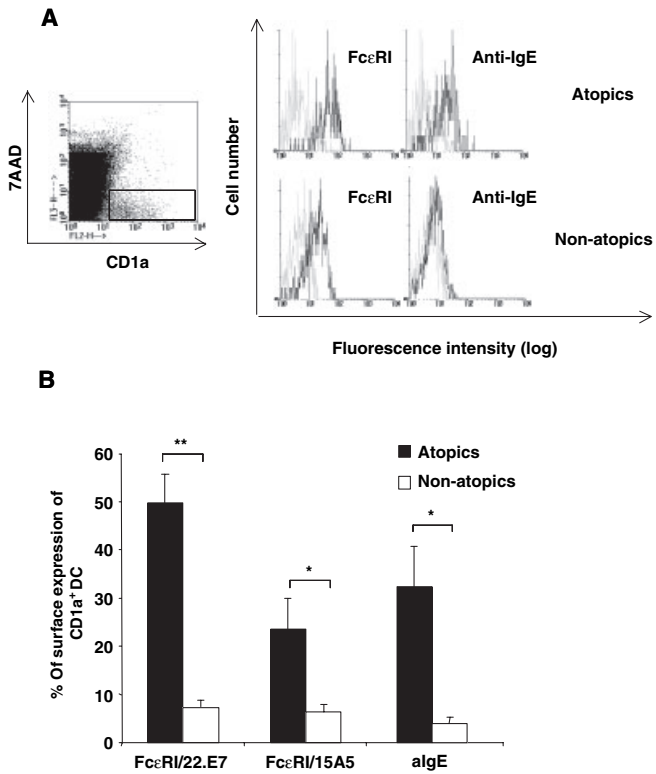


Figure 2. Nasal mucosal myeloid dendritic cell (nmDC) constitutively express high affinity receptor for immunoglobulin E (FcεRI). (A) Single cells suspension was stained for flow cytometry. nmDC were detected by their CD1a expression and gated manually. Dead cells were excluded by 7-amino-actinomycin-D dye. nmDC of atopic displayed highest amount of FcεRI and surface-bound IgE. (B) By using one antibody directed against a site different from IgE-binding to FcεRI (22.E7) and one antibody directed against the binding site of IgE on to FcεRI (15A5) we could demonstrate that FcεRI on nmDC is partly occupied by IgE (*n* = 20).

nmDC for FcεRI expression (9). We could thereby detect low amounts of FcεRI on nmDC of nonatopic donors, while high surface expression of FcεRI was demonstrated on nmDC of atopic individuals. In parallel, only low amounts of IgE molecules were detectable on the surface of nmDC of nonatopic donors while nmDC of atopic patients displayed high amounts of IgE (Fig. 2A). With the help of a mAb competing with the IgE-binding site of FcεRI (15A5) we could show that over 50% of the IgE-binding sites of FcεRI on the surface of nmDC isolated from atopics were occupied with IgE molecules (Fig. 2B). Moreover, surface expression of FcεRI on the surface of nmDC not only correlated with the total serum IgE level of the volunteers but also with the amount of IgE bound to the cell surface of these cells (Fig. 2C).

From this set of experiments we concluded that just as omDC, nmDC constitutively express FcεRI with the highest expression observed in atopic individuals.

Oral mucosal CD1a⁺ DC express higher levels of FcεRI

As one common feature of nmDC and omDC was reflected by a constitutive FcεRI expression we next compared its expression density on both DC types in respect to the atopic status of the donors. We could thereby show that omDC of atopic as well as nonatopic donors displayed a higher density of FcεRI than nmDC with the highest difference in atopic individuals (Fig. 3).

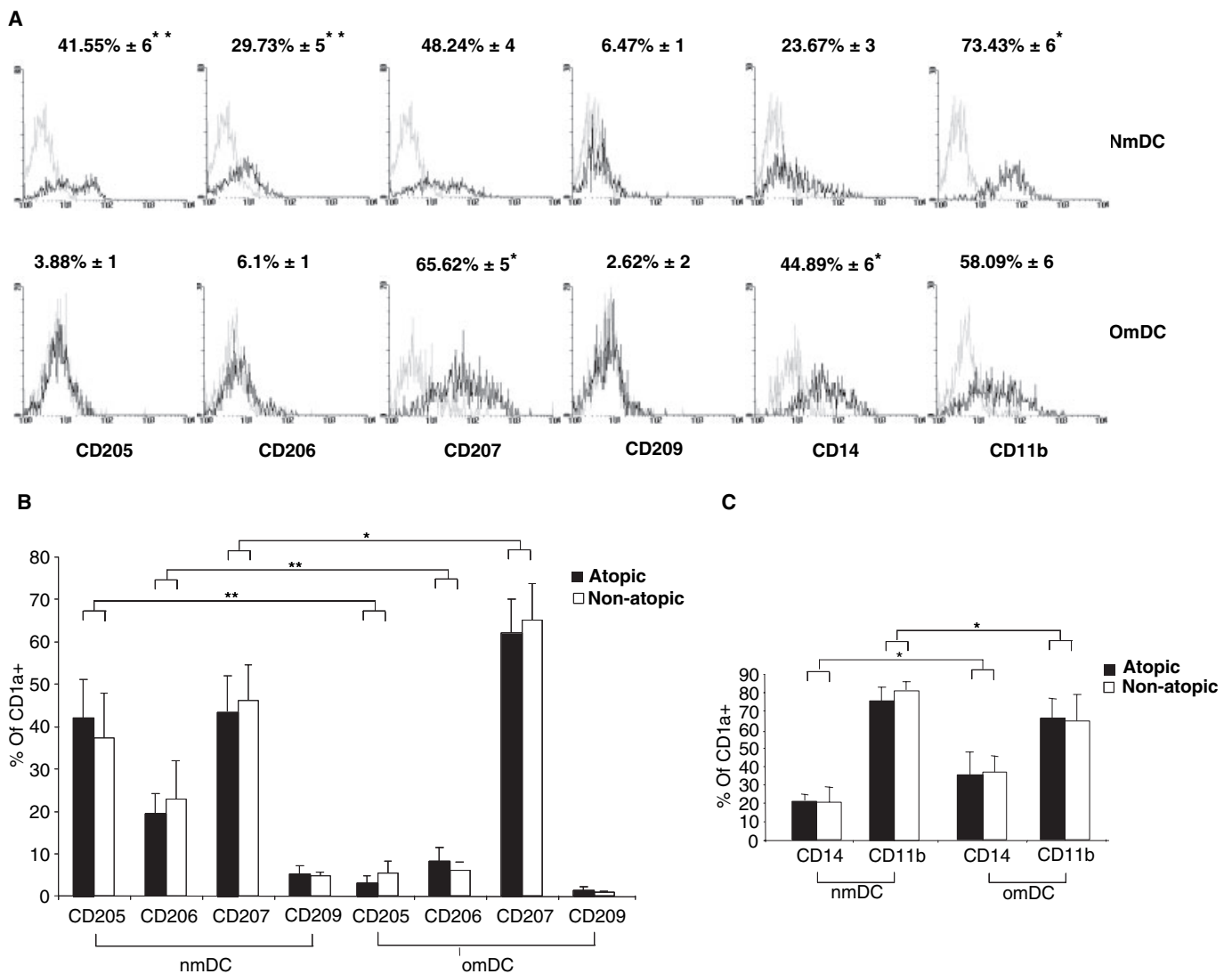


Figure 3. C-type lectins and myeloid markers on nasal mucosal myeloid dendritic cell (nmDC) and oral mucosal myeloid DC (omDC). Single cell suspension of oral and nasal mucosa was stained for flow cytometry. omDC and nmDC were detected by their CD1a expression and gated manually. Dead cells were excluded by 7-amino-actinomycin-D dye. (A) nmDC expressed higher amounts of CD205, CD206 and CD11b while omDC displayed higher CD207 and CD14 expression. (B) No significant differences could be seen between atopics and non-atopics in C-type lectin expression as well as (C) expression of CD14 and CD11b (nmDC: $n = 10$, omDC: $n = 10$).

From these data we concluded that although FcεRI expression is a common feature of nmDC and omDC both mucosal DC types still differ by the expression density of this immunoglobulin receptor.

Diverse expression of costimulatory and MHC molecules on nmDC and omDC

We could show recently that compared with skin epithelium DC, omDC express significant amounts of costimulatory molecules CD40, CD80 and CD86 as well major MHC class I and II molecules so that we next compared their expression with nmDC (9). We could thereby demonstrate that nmDC also express significant amounts of costimulatory molecules CD80, CD86 and MHC class I and II. Furthermore, nmDC and omDC of atopic individuals expressed more costimulatory molecule CD40 than nonatopics. In comparison nmDC and omDC equally express the costimulatory molecule CD40 while nmDC express more CD80 and omDC more CD86. In view of MHC class molecules we could detect more MHC class I and II on omDC than nmDC. Both DC types showed equal CD83 expression (Fig. 4).

In conclusion, nmDC and omDC further differ in expression of costimulatory as well as MHC class molecules. Moreover, CD40 was enhanced on nmDC and omDC of atopic individuals.

nmDC and omDC differentially express lectins and myeloid markers

While the nasal mucosa is outlined by ciliated epithelium the oral mucosa is build up by a nonkeratinized stratified

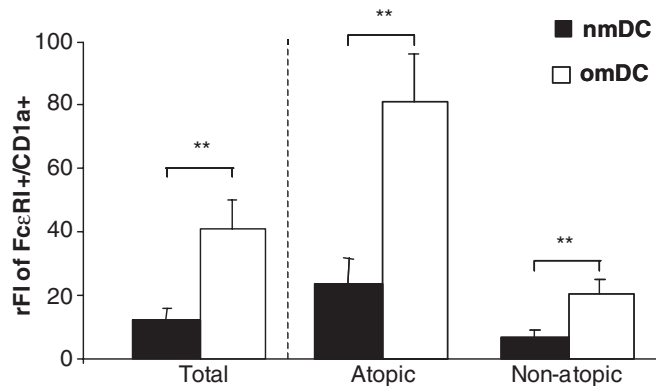


Figure 4. Oral myeloid dendritic cell express higher amounts of high affinity receptor for immunoglobulin E (FcεRI). Single cell suspension of oral and nasal mucosa was stained for flow cytometry. Oral mucosal myeloid DC (omDC) and nasal mucosal myeloid DC (nmDC) were detected by their CD1a expression and gated manually. Dead cells were excluded by 7-amino-actinomycin-D dye. Results are shown in relative fluorescence index (rFI) to compare the density of FcεRI on DC. Considering all samples (nmDC: $n = 20$, omDC: $n = 20$) density of FcεRI was highest on omDC. The discrepancy was greatest when omDC of atopics ($n = 10$) was compared with nmDC of atopics ($n = 10$).

squamous epithelium so that we subjected nmDC and omDC to detailed analysis of lineage-specific markers. We could thereby detect a significant higher expression of C-type lectins DEC205/CD205 and mannose receptor/CD206 on nmDC compared with omDC while the latter in contrast to nmDC expressed higher amounts of Langerhans' cell (LC)-specific marker langerin/CD207 (Fig. 5A). Furthermore, nmDC expressed very low amounts of DC-SIGN/CD209 while it was virtually absent on omDC. Moreover, while both DC types expressed the myeloid lineage markers CD11b and lipopolysaccharide (LPS) receptor CD14 nmDC displayed higher CD11b and omDC higher CD14 expression. No differences in C-type lectins and myeloid marker expression could be detected comparing nmDC and omDC from atopics and nonatopics (Fig. 5B).

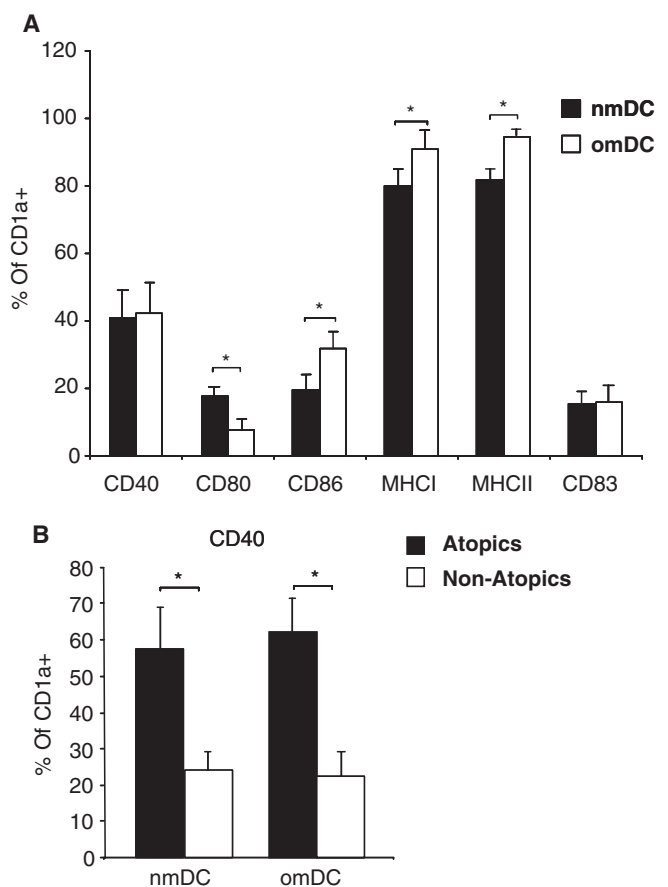


Figure 5. Expression of costimulatory and major histocompatibility complex (MHC) class I and II molecules. Single cell suspension of oral and nasal mucosa was stained for flow cytometry. Oral mucosal myeloid DC (omDC; $n = 10$) and nasal mucosal myeloid DC (nmDC; $n = 10$) were detected by their CD1a expression and gated manually. Dead cells were excluded by 7-amino-actinomycin-D dye. (A) CD80 expression was higher on nmDC while omDC express more CD86, MHC I and MHC II molecules. Both DC types express equal amounts of CD40. (B) CD40 expression on nmDC and omDC was higher in samples of atopic individuals compared with nonatopics.

From this set of experiments we conclude that although both DC types share the feature of FcεRI expression nmDC displayed more markers associated with interstitial or dermal DCs (18) while omDC appeared to belong more to the LC family.

Discussion

The DC not only play a central role in the development but also in the treatment of atopic diseases such as allergic rhinitis. In this context within the recent years nasal and sublingual immunotherapy have been demonstrated to be an alternative route to conventional subcutaneous immunotherapy (3). Nevertheless only limited data exist about DC phenotype within the nasal and oral mucosa of atopic and nonatopic individuals. In this report, we studied and compared for the first time nasal and oral mucosal CD1a⁺ mDC of atopic and nonatopic individuals. We could thereby show that both DC types share the feature of FcεRI expression but differ in view of (i) surface density of FcεRI, (ii) lineage-specific as well as (iii) myeloid marker, and (iv) costimulatory and MHC class expression.

As we could demonstrate recently constitutive FcεRI expression represents a unique feature of omDC compared with normal skin (9). Nevertheless in this study we could demonstrate that nmDC show the same distribution pattern of FcεRI. It is seductive to speculate that FcεRI expression might represent a common feature on mucosal DC in general. This might be related to the microenvironment of mucosal tissue as it could be shown that microenvironment is a critical factor for stabilization of FcεRI on DC *in vitro* (10). Furthermore, it is well accepted that IgE binding to FcεRI also stabilizes its expression (10, 11, 19), which could explain the increased expression of FcεRI on omDC and nmDC of atopic individuals who display increased IgE levels (20). The FcεRI-bearing DC in nasal as well as oral mucosa might bind allergen-specific IgE via FcεRI and thereby play not only a role in the uptake and presentation of allergens but also represent important target cells for antiallergic therapeutic strategies (13). Taken together we provide evidence that nmDC are very similar to omDC in terms of FcεRI expression. On the contrary, we could show that these two DC types completely differ in lineage marker expression. Especially C-type lectins CD205 and CD206 are highly expressed by nmDC. These structures have

been shown to be associated with dermal DC but classical epidermal DC – namely LC lack these C-type lectins (5, 18). The LC-associated lectin CD207 (21) was expressed by both DC types although it was mostly expressed by omDC. This results in the conclusion that omDC represent a more homogenous DC cell population belonging to LC family while the nmDC population appears to be more heterogenous comprising LC and dermal DC.

Another interesting result in this study was the detection of LPS receptor CD14 on nmDC and omDC of which the latter expressed the highest amounts. Furthermore, it has been reported that dosage LPS is involved in modulating Th1 and Th2 immune responses in mice whereby nasal exposure of high LPS doses combined with ovalbumin (OVA) resulted in Th1 while low doses of LPS combined with OVA led to a Th2 immune response (22). A shift from an allergy-tended Th2 to allergy-protecting Th1 immune response is thought to be one mechanism important for successful immunotherapy (1). In view of the resistant flora within the oral cavity it is tempting to speculate that microbial products may critically modulate the immune system towards allergy protection represented either by Th1 type response or tolerance. This might be important in the selection of potential adjuvants for local immunotherapy. In this context, the high expression of costimulatory molecule CD40 on nmDC and omDC might also be relevant for immunotherapy because it has been reported that interaction between CD40 and its ligand (CD40L) are capable of inducing systemic unresponsiveness (23). Interestingly, nmDC and omDC of atopic individuals displayed a higher CD40 expression than nonatopics although we could not detect an altered allogeneic immune response between atopic and nonatopic individuals.

In conclusion, nasal and oral mucosa comprises distinctive DC types which share FcεRI expression but differ in their further phenotype and functional properties. The diverse expression of C-type lectins might result from different mucosal microenvironment and anatomic structure. The expression of CD14 on nmDC and especially omDC might be relevant in search of potent adjuvants for local immunotherapy.

Acknowledgments

This work was supported by grants from BONFOR and Bencard.

References

1. Norman PS. Immunotherapy: 1999–2004. *J Allergy Clin Immunol* 2004;**113**:1013–1023.
2. Novak N, Allam JP, Betten H, Haberstok J, Bieber T. The role of antigen presenting cells at distinct anatomic sites: they accelerate and they slow down allergies. *Allergy* 2004;**59**:5–14.
3. Canonica GW, Passalacqua G. Noninjection routes for immunotherapy. *J Allergy Clin Immunol* 2003;**111**:437–448.
4. Novak N, Kraft S, Bieber T. IgE receptors. *Curr Opin Immunol* 2001;**13**:721–726.

5. Geijtenbeek TB, Engering A, Van KY. DC-SIGN, a C-type lectin on dendritic cells that unveils many aspects of dendritic cell biology. *J Leukoc Biol* 2002;**71**:921–931.
6. Currie AJ, Stewart GA, McWilliam AS. Alveolar macrophages bind and phagocytose allergen-containing pollen starch granules via C-type lectin and integrin receptors: implications for airway inflammatory disease. *J Immunol* 2000;**164**:3878–3886.
7. Ying S, Barata LT, Meng Q, Grant JA, Barkans J, Durham SR et al. High-affinity immunoglobulin E receptor (Fc epsilon RI)-bearing eosinophils, mast cells, macrophages and Langerhans' cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *Immunology* 1998;**93**:281–288.
8. Granucci F, Ricciardi-Castagnoli P. Interactions of bacterial pathogens with dendritic cells during invasion of mucosal surfaces. *Curr Opin Microbiol* 2003;**6**:72–76.
9. Allam JP, Novak N, Fuchs C, Asen S, Berge S, Appel T et al. Characterization of dendritic cells from human oral mucosa: a new Langerhans' cell type with high constitutive FcepsilonRI expression. *J Allergy Clin Immunol* 2003;**112**:141–148.
10. Novak N, Kraft S, Haberstok J, Geiger E, Allam P, Bieber T. A reducing microenvironment leads to the generation of FcepsilonRI high inflammatory dendritic epidermal cells (IDEC). *J Invest Dermatol* 2002;**119**:842–849.
11. Allam JP, Klein E, Bieber T, Novak N. Transforming growth factor-beta1 regulates the expression of the high-affinity receptor for IgE on CD34 stem cell-derived CD1a dendritic cells in vitro. *J Invest Dermatol* 2004;**123**:676–682.
12. Smurthwaite L, Durham SR. Local IgE synthesis in allergic rhinitis and asthma. *Curr Allergy Asthma Rep* 2002;**2**:231–238.
13. Babu KS, Arshad SH, Holgate ST. Anti-IgE treatment: an update. *Allergy* 2001;**56**:1121–1128.
14. Arikian C, Bahceciler NN, Deniz G, Akdis M, Akkoc T, Akdis CA et al. Bacillus Calmette-Guerin-induced interleukin-12 did not additionally improve clinical and immunologic parameters in asthmatic children treated with sublingual immunotherapy. *Clin Exp Allergy* 2004;**34**:398–405.
15. Kraft S, Wessendorf JH, Hanau D, Bieber T. Regulation of the high affinity receptor for IgE on human epidermal Langerhans cells. *J Immunol* 1998;**161**:1000–1006.
16. Jahnsen FL, Lund-Johansen F, Dunne JF, Farkas L, Haye R, Brandtzaeg P. Experimentally induced recruitment of plasmacytoid (CD123high) dendritic cells in human nasal allergy. *J Immunol* 2000;**165**:4062–4068.
17. Lambrecht BN. Dendritic cells and the regulation of the allergic immune response. *Allergy* 2005;**60**:271–282.
18. Ebner S, Ehammer Z, Holzmann S, Schwingshackl P, Forstner M, Stoitzner P et al. Expression of C-type lectin receptors by subsets of dendritic cells in human skin. *Int Immunol* 2004;**16**:877–887.
19. Borkowski TA, Jouvin MH, Lin SY, Kinet JP. Minimal requirements for IgE-mediated regulation of surface Fc epsilon RI. *J Immunol* 2001;**167**:1290–1296.
20. Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF et al. Revised nomenclature for allergy for global use: report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol* 2004;**113**:832–836.
21. Valladeau J, Duvert-Frances V, Pin JJ, Zutter-Dambuyant C, Vincent C, Massacrier C et al. The monoclonal antibody DCGM4 recognizes langerin, a protein specific of Langerhans cells, and is rapidly internalized from the cell surface. *Eur J Immunol* 1999;**29**:2695–2704.
22. Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, Toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002;**196**:1645–1651.
23. Kweon MN, Fujihashi K, Wakatsuki Y, Koga T, Yamamoto M, McGhee JR et al. Mucosally induced systemic T cell unresponsiveness to ovalbumin requires CD40 ligand-CD40 interactions. *J Immunol* 1999;**162**:1904–1909.