Characterization of FcεRI-bearing CD123$^+$ blood dendritic cell antigen-2$^+$ plasmacytoid dendritic cells in atopic dermatitis

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Background: The high-affinity receptor for IgE (FcεRI) on myeloid dendritic cells has been shown to play a major role in atopic dermatitis (AD). Plasmacytoid dendritic cells (pDCs), which are instrumental in the defense of viral infections, are present in reduced amounts in the skin of patients with AD, which is characterized by a high susceptibility to viral infections.

Objective: We explored phenotypical and functional characteristics of pDC in the peripheral blood of patients with AD and healthy individuals.

Methods: Blood dendritic cell antigen-2$^+$CD123$^+$ pDCs were enriched from the peripheral blood of patients with AD and studied in functional assays.

Results: Skin-homing molecules such as cutaneous lymphocyte antigen and L-selectin CD62L were expressed in lower levels on pDCs of patients with AD. pDCs expressed high amounts of IgE-occupied FcεRI. Further, FcεRI aggregation on pDCs impaired the surface expression of MHC I and II, induced the production of IL-10, and enhanced the apoptosis of pDCs. Importantly, FcεRI preactivated pDC produced less IFN-α and IFN-β after stimulation with CpG motifs and enhanced the outcome of immune responses of the T_H2 type.

Conclusion: From these data, we conclude that FcεRI-bearing pDCs from patients with AD (1) are different from pDCs of healthy individuals, (2) might be important in the pathophysiology of AD, and (3) contribute to the enhanced susceptibility of patients with AD to viral infections. (J Allergy Clin Immunol 2004;114:364-70.)

Key words: *Plasmacytoid dendritic cells, IgE, FcεRI, atopic dermatitis, IFN-α/β*

Abbreviations used
AD: Atopic dermatitis
BDCA: Blood dendritic cell antigen
CLA: Cutaneous lymphocyte antigen
DC: Dendritic cell
FITC: Fluorescein isothiocyanate
mDC: Myeloid dendritic cell
MFI: Mean fluorescence intensity
pDC: Plasmacytoid dendritic cell
RFI: Relative fluorescence intensity

In the human immune system, 2 distinct types of dendritic cells (DCs) can be characterized: myeloid DC (mDC or DC1), and lymphoid DC (DC2).[1] The latter contain 2 subpopulations: the CD123^{dim} CD11c^{bright} DCs with a rather monocytic appearance, and the CD123^{bright} CD11c$^-$ blood DCs, which display a plasmacytoid morphology.[2,3] In the peripheral blood, the expression of the blood dendritic cell antigen (BDCA)–2 is strictly associated with CD123^{bright} CD11c$^-$ plasmacytoid DC (pDC).[4]

It has been believed for a long time that these CD123$^+$ BDCA-2 pDCs lack Fc receptors such as the high affinity receptor for IgE (FcεRI), but more recently, the presence of FcεRI has been demonstrated on the surface of pDCs of patients with allergic asthma.[5,6] So far, detailed analyses of pDCs in the peripheral blood of patients with atopic dermatitis (AD) are missing. In the past, FcεRI was considered a structure that was a unique feature of the effector cells of anaphylaxis such as mast cells and basophils.[7] This assumption was modified more than 10 years ago, when a trimeric form of FcεRI consisting of an IgE-binding α-chain and a γ-chain dimer bearing the immunoreceptor tyrosine-based activation motifs for signal transduction, lacking the β-chain, was discovered on mDCs of patients with atopic diseases.[8,9] FcεRI expression on these cells was significantly higher in atopic individuals and was shown to be distinctly regulated in atopic versus nonatopic volunteers.[10] In addition, FcεRI-bearing monocytes and DCs can be directly activated by interactions with multivalent allergens, resulting in enhanced allergen presentation and

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synthesis of proinflammatory cytokines.[11] Beside its role in the amplification of allergic-inflammatory immune responses, FcεRI on the surface of antigen-presenting cells has been suggested to be involved in the induction of anti-inflammatory and rather tolerogenic immune mechanisms. Functional clues about this phenomenon have come from the observation that FcεRI aggregation on the surface of monocytes leads to the rapid release of the tolerogenic cytokine IL-10.[12]. Moreover, cross-linking of FcεRI on monocytes also induces the production of the enzyme indoleamine 2,3-dioxygenase, which is the rate-limiting enzyme in the catabolism of the essential amino acid tryptophan and is involved in the regulation of T-cell responses.[13] In the current study, FcεRI$^+$ pDCs from healthy individuals and patients with AD were subjected to detailed comparative explorations whereby significant differences could be highlighted that might provide a first explanation for the high susceptibility of patients with AD to viral infections.

METHODS

For the section "Reagents," please see the ancillary material in the Journal's Online Repository at www.mosby.com/jaci.

Patients and diagnostic criteria

A total of 25 healthy volunteers (age, 39.92 years ± 13.0 years; serum IgE, 22.09 kU/L ± 31.5 kU/L) and 50 patients with AD (age, 39.25 years ± 15.71 years; serum IgE, 2.118 kU/L ± 1082 kU/L) were investigated. The protocol was approved by the local ethical committee. AD was diagnosed according to the criteria of Hanifin and Rajka.[14] Patients on UV therapy, on systemic or local glucocorticosteroid treatment, taking antihistamines, or with viral infections were excluded from this study. Total IgE in the sera of these patients was analyzed with the Immunlite 2000 System (DPC Biermann Diagnostika GmbH, Bad Nauheim, Germany).

Cell isolation

Human PBMCa were obtained from heparinized blood (50-100 mL) by density gradient centrifugation. After the blood was diluted 3 times with PBS, 25 mL suspended cells was overlain on 15 mL Lymphoprep (Progen, Heidelberg, Germany). PBMCs were isolated as interface cells after density gradient centrifugation (20 minutes at 900*g* at room temperature). To reduce residual platelets, the recovered cells were washed twice in PBS and recovered in PBS supplemented with 1% BSA and 5 mmol/L EDTA. For some experiments, isolation of BDCA-4$^+$ pDC was performed with the AUTOMACS technique (Miltenyi Biotec, Bergisch Gladbach, Germany) by using the BDCA-4 dendritic cell isolation kit (Miltenyi Biotec). The purity of the isolated pDC (lineage-negative, MHC II–positive, BDCA-2$^+$/BDCA-4$^+$, CD123$^+$ cells) was >95%. Viability was >95% as determined by 7-aminoactinomycin D staining.

For the isolation of autologous naive T cells, PBMCs were isolated from heparinized blood of the same donor by standard gradient centrifugation with Lymphoprep. PBMCs were harvested from the interface, washed twice, and resuspended in PBS supplemented with 5 mol/L EDTA and 0.5% human serum albumin. Purified naive CD4/CD45A$^+$ T cells were obtained directly from PBMCs by positive selection with paramagnetic beads or by negative selection and high-gradient magnetic sorting by using the AUTOMACS technique as described in the manufacturer's instructions. The purity of the enriched naive T cells evaluated by flow cytometry was >95%.

Flow-cytometric analysis

Immunolabeling for phenotyping was performed as previously reported.[12] Intracellular staining for cytokines and chemokines was performed according to the manufacturer's instructions (PharMingen, Hamburg, Germany). Briefly, cells cultured in the presence of Monesin were fixed with 4% paraformaldehyde for 10 minutes, washed in PBS supplemented with 1% FCS and 0.1% sodium azide, and incubated for 30 minutes with 1 μg/mL FITC-conjugated CD1a. Then the cells were washed twice in PBS containing 1% FCS, 0.1% sodium azide, and 0.1% saponin. The cells were incubated with phycoerythrin-labeled or FITC-labeled mAb against cytokines. After washing with PBS, cells were analyzed on a FACS Calibur (BD) as described in detail elsewhere.[12] As a control, cells were also stained with corresponding phycoerythrin-labeled isotype-matched control Ig. All incubations and washes were performed at 4°C. Results are expressed as the percentage of positive cells compared with isotype control, and the relative fluorescence intensity (RFI) was calculated from mean fluorescence intensity (MFI) as follows: RFI = (MFI [cytokine/chemokine] – MFI [isotype control])/MFI (isotype control).

Amplification of mRNA and analysis of FcεRI transcripts

Plasmacytoid dendritic cells were enriched from the peripheral blood as described. Then, total RNA was extracted from highly purified PBMCs by using the RNA Mini Kit (Quiagen GmbH, Hilden, Germany), following the manufacturer's instructions. RT reactions were performed by using 1 μg RNA. Denaturation at 94°C for 40 seconds was followed by primer annealing at 55°C for 30 seconds and extension at 72°C for 30 seconds. A final extension phase of 5 minutes was added. Specific primer sequences for each gene were as follows: human β-actin: sense, 5′-GAG CGG GAA ATC GTG CGT GAC ATT-3′; antisense, 5′-GAT GGA GTT GAA GGT AGT TTC GTG-3′ (240-bp fragment); human FcεRIα: sense, 5′-CTG TTC TTC GCT CCA GAT GGC GT-3′; antisense, 5′-TAC AGT AAT GTT GAG GGG CTC AG-3′ (536-bp fragment); human FcεRIβ: sense, 5′-GGA CAC AGA AAG TAA TAG GAG AG; antisense, 5′-GAT CAG GAT GGT AAT TCC CGT T (446-bp fragment); human FcεRIγ: sense, 5′-CCA GCA GTG GTC TTG CTC TTA C-3′; antisense, 5′-GCA TGC AGG CAT ATG TGA TGC C-3′ (338-bp fragment). Amplification was performed on a Perkin-Elmer Gene Amp PCR System 9600 thermocycler (Applied Biosystems, Weiterstadt, Germany). The PCR cycle numbers for the amplification of the respective cDNAs were 25 for β-actin and 30 for FcεRIα, FcεRIβ, and FcεRIγ. Specific PCR fragments were separated on a 1% agarose gel and visualized by using ethidium bromide staining. The PCR products were evaluated semiquantitatively by comparing the ratio of the specific products versus the β-actin band by digital image analysis by using the WinCam system (Cybertech, Berlin, Germany).

FcεRI-mediated activation of pDC

Plasmacytoid dendritic cells were washed and incubated at 2.5×10^5 cells/mL in 24-well flat-bottom culture plates in medium containing human myeloma IgE (1 μg/mL) for 1 hour at 37°C. Excessive IgE was removed, and surface-bound IgE was cross-linked by addition of medium containing 50 μg/mL rabbit antihuman IgE antibody. Alternatively, cells were activated by direct ligation of FcεRI by using mAb 22E7 or isotype-matched control mIgG1 and goat antimouse Ig $(F(ab')^2$ as previously described.[15] As a control, antihuman IgE antibody or goat-antimouse Ig $(F(ab)^2$ alone were added to the cells to exclude an unspecific activation of the cells.

Polarization of T cells

To assess cytokine secretion by T cells after activation by pDC, acid stripping to remove the IgE molecules from the cell surface was performed as described previously.[16] FcεRI was loaded with Bet v 1 specific chimeric IgE (1 μg/mL), washed and cross-linked with recombinant Bet v 1 (10 μg/mL), fed with 20 ng/mL recombinant IL-3, and cocultured with autologous naive $CD4^+CD45RA^+$ T cells.

T cells were then stimulated with 50 ng/mL phorbol 12-myristate 13-acetate (Sigma GmbH, Munich, Germany) plus 500 ng/mL ionomycin (Sigma) for 6 hours. Monensin (2 μmol/L; Sigma) was added during the final 4 hours. Cells were then stained with mAb against CD3 (PerCP) and fixed with 1% paraformaldehyde in PBS and permeabilized and stained with mAb against IL-4 (phycoerythrin) and IFN-γ (FITC).

IFN-α and IFN-β production of pDC

Plasmacytoid dendritic cells were stimulated with 10 μg/mL anti-FcεRI mAb $(F(ab')^2$ 22E7 for 1 hour at 37°C. After washing with culture medium, 20 μg/mL goat-antimouse IgG $(Fab')^2$ fragment (Jackson Immuno Research Laboratories, West Grove, Pa) was added.

The sequence of CpG-A (ODN 2216) was 5′GGggacga:tcgtcGGGGGg33′ and has been described previously.[17] Intracellular cytokine staining in pDCs was performed after a total of 18 hours of stimulation, as described previously.[17]

Statistical analysis

For statistical evaluation, Wilcoxon or Mann-Whitney *U* tests and Pearson linear regression analysis were performed with SPSS 10.0 for Windows (CRN Inc, Chicago, Ill). Results are given in mean percentages ± SEMs. A *P* value of $< .05$ was considered significant. The Spearman correlation coefficient was chosen for the overall correlation of the total serum IgE levels and the FcεRI/IgE surface expression of pDCs.

RESULTS

$CD123^+BDCA\text{-}2^+$ pDCs in the peripheral blood of patients with AD display different phenotypical characteristics

First, we evaluated phenotypical characteristics of $CD123^+BDCA\text{-}2^+$ pDCs in the peripheral blood of patients with AD and healthy, nonatopic volunteers in detail by flow cytometry. $BDCA\text{-}2^+CD123^+$ pDCs freshly isolated from the blood of patients with AD expressed higher amounts of the costimatory molecule CD80 and the MHC I molecule on their cell surface than $BDCA\text{-}2^+CD123^+$ pDCs of healthy, nonatopic volunteers (Fig 1). In contrast, surface expression of L-selectin CD62L and cutaneous lymphocyte antigen (CLA), which play a major role in the recruitment process of pDCs to the skin, were expressed in significantly lower amounts on the surface of $BDCA\text{-}2^+CD123^+$ pDCs in the peripheral blood of patients with AD (Fig 1).

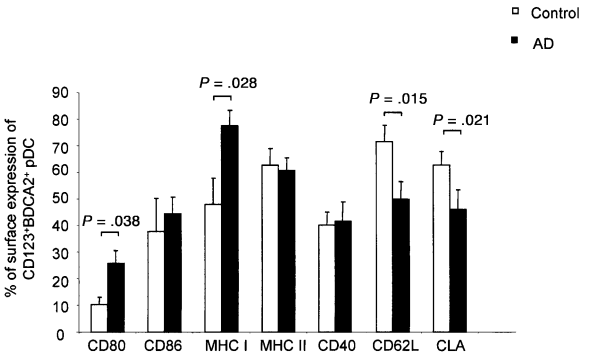

FIG 1. Phenotypic differences of pDCs obtained from the peripheral blood of patients with AD and healthy controls. The percentage of surface expression on $CD123^+$ $BDCA\text{-}2^+$ pDCs of the peripheral blood from healthy control volunteers *(white bars)* and patients with AD *(black bars)* is depicted on the y-axis; n = 10 for each group.

$BDCA\text{-}2^+CD123^+$ pDCs express the trimeric variant of the high-affinity receptor for IgE

Further, we found that pDCs of patients with AD displayed significantly higher surface levels of the high-affinity receptor for IgE (FcεRI) than pDCs from healthy control individuals. In addition, in patients with AD, most of the IgE binding sites of FcεRI on the surface of pDCs were occupied with IgE molecules (Fig 2, *A*). The FcεRI surface expression of pDCs highly correlated with the total IgE serum level of these patients ($r = 0.446$; $P = .0001$).

To analyze the exact structure of FcεRI on pDCs, we evaluated the surface expression of the FcεRIα-chain in parallel with the intracellular pool of the FcεRIα-chain (Fig 2, *B*) and the intracellular amount of the FcεRIγ-chain (Fig 2, *C*) by flow-cytometric staining. For this purpose, we permeabilized the cells and evaluated the intracellular FcεRIα and FcεRIγ-pool. We could show that the intracellular level of the FcεRIα-chain and the FcεRI-γ chain was high in $CD123^+BDCA\text{-}2^+$ pDCs. To confirm these results on the mRNA level and to reveal whether FcεRI on pDCs displays the trimeric (α2γ) or tetrameric (αβ2γ) variant, we performed RT-PCR experiments. In these experiments, we could show transcripts of the FcεRIα-chain and FcεRIγ-chain, whereas transcripts of the FcεRIβ-chain were not detectable in highly purified $CD123^+BDCA\text{-}2^+$pDCs enriched from the peripheral blood (Fig 2, *D*). From these data, we conclude that FcεRI on pDCs, similar to mDCs, consists of the trimeric FcεRIα2γ-variant.

MHC molecules and the costimulatory molecule CD40 are downregulated in consequence of FcεRI engagement

Aggregation of FcεRI on the surface of pDCs of patients with AD led to phenotypic alterations of the surface marker profile of these cells. The surface expression of MHC class I and II and the costimulatory molecule CD40 decreased significantly after FcεRI engagement of pDCs of patients with AD, whereas the surface expression of the costimulatory molecules CD80 and CD86 did not change significantly (Fig 3).

FcεRI activation induces the production of IL-10 and apoptosis of pDCs

It has been shown previously that FcεRI aggregation induces the release of anti-inflammtory signals such as IL-10 on mDCs. We next investigated the amount of IL-10

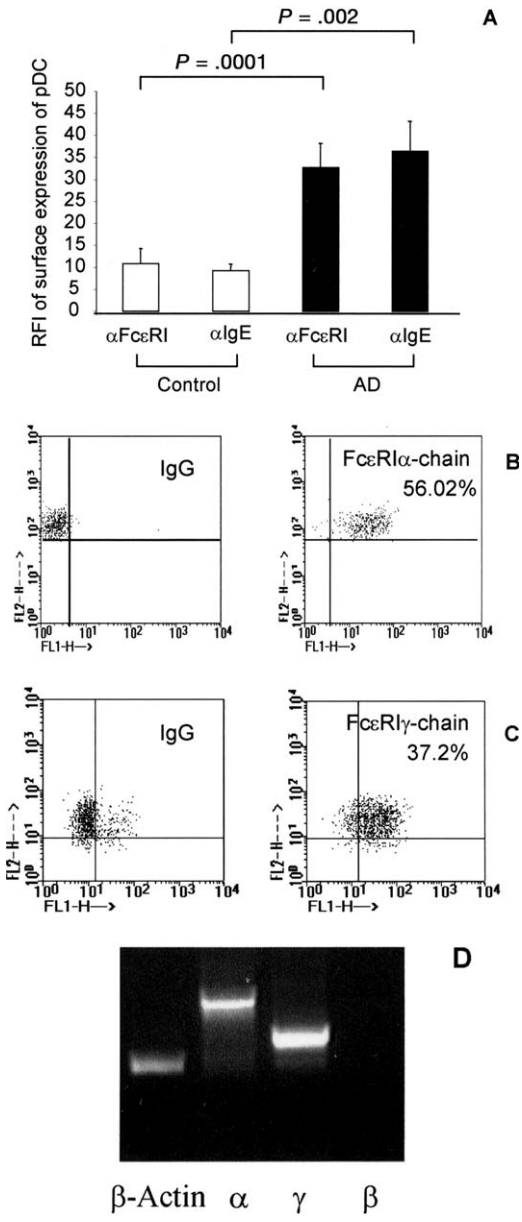

FIG 2. Surface expression of the trimeric variant of FcεRI on pDCs is higher in patients with AD than in healthy, nonatopic volunteers. **A,** FcεRI surface expression and IgE binding is higher in patients with AD. RFI of the surface expression is depicted on the y-axis (n = 17, controls; n = 26, patients with AD). **B,** $CD123^+BDCA\text{-}2^+$ pDCs express enhanced intracellular amounts of the FcεRI-α chain and the FcεRI-γ chain **(C). D,** Transcripts of the FcεRIα-chain and FcεRIγ-chain are detectable on the RNA level of pDCs, whereas transcripts of the FcεRI-β chain are completely absent. This is 1 representative experiment of 3.

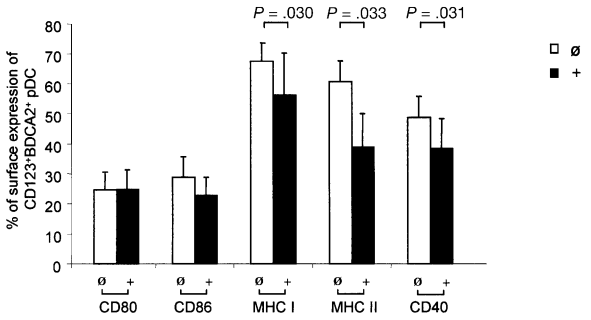

FIG 3. Costimulatory molecules and MHC molecules are downregulated in consequence of FcεRI aggregation on pDCs of patients with AD. **A,** Surface expression of costimlatory molecules and MHC molecules on pDCs with FcεRI engagement via IgE and rabbit antihuman IgE *(black bars/+)* and unstimulated *(white bars/Ø)* is depicted on the y-axis. The percentage of $BDCA\text{-}2^+CD123^+$ pDCs expressing CLA **(B)** and CD62L **(C)** of nonatopic controls *(white bars/Control)* and patients with AD *(black bars/AD)* is depicted on the y-axis; n = 17 nonatopic controls; n = 18 patients with AD.

produced by pDCs after FcεRI aggregation. We could show that in consequence of FcεRI engagement, pDCs produce significantly higher amounts of IL-10 within 18 hours of stimulation (Fig 4, *A-C*). Because IL-10 has been shown to influence the viability of pDCs, we studied the rate of apoptosis of pDCs in consequence of FcεRI cross-linking. We found that pDCs that underwent FcεRI aggregation showed an enhanced level of apoptosis revealed by annexin V staining in comparison with pDCs that were left unstimulated (Fig 4, *D*). Interestingly, addition of 1 μg/mL neutralizing antibody against IL-10 that was added immediately after cross-linking to the cell culture was able to prevent pDCs from undergoing FcεRI-induced apoptosis (Fig 4, *D*).

Allergen challenge of pDCs leads to a higher polarization of T cells into T cells of the T_H2 type

It is well known that the ability to prime naive T cells is a unique feature of DCs. Thus, we designed the next series of experiments to evaluate putative differences in the capacity of pDCs to polarize naive T cells with and without allergen challenge. For this purpose, constitutive IgE bound to pDCs was removed via acid stripping. Afterward, pDCs were preloaded with IgE Bet v 1 allergen complex and coincubated with autologous naive T cells. After 6 days of coincubation, the quality and quantity of the production of the T_H2 cytokine IL-4 and the T_H1 cytokine IFN-γ of the cocultured T cells were evaluated in functional assays.

In summary, pDCs loaded with Bet v 1 allergen–IgE immune complexes induced T cells of the T_H2 type, producing higher amounts of IL-4 and lower levels of IFN-γ than T cells cocultured with untreated pDCs (Fig 5, *A*).

FcεRI-activated pDCs produce lower amounts of IFN-α and IFN-β

Because pDCs have been shown to produce IFN-α and IFN-β after activation with immunostimulatory agents such as CpG motifs and the main antiviral activity of pDCs is mediated via the production of these IFNs, we studied the capacity of FcεRI-activated pDCs to produce IFN-α and INF-β after challenge with CpG motifs.

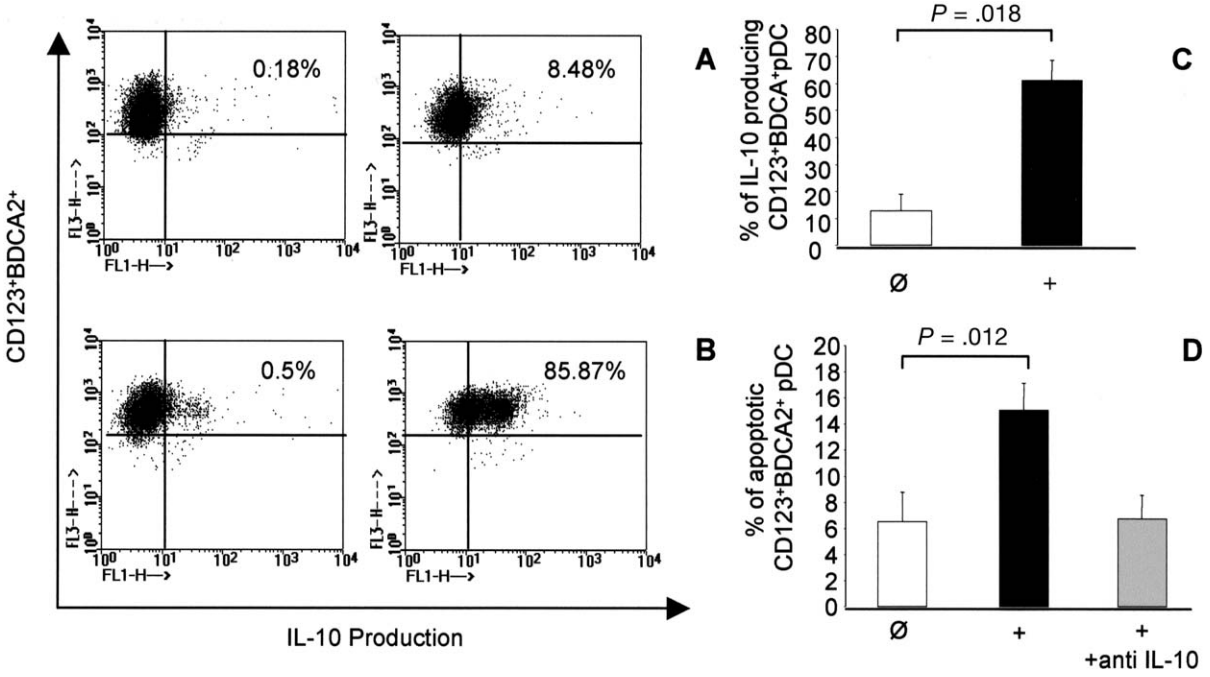

FIG 4. Enhanced production of IL-10 after FcεRI engagement on pDCs. The intracellular amount of IL-10 produced by pDCs is enhanced 18 hours after stimulation of pDCs via FcεRI **(B)** in comparison with the unstimulated control **(A)**. In parallel, the amount of IL-10 released in the supernatant by pDCs is significantly enhanced in consequence of FcεRI engagement via IgE and rabbit antihuman IgE **(C)**; *white bars/Ø* = without FcεRI engagement; *black bars/+* = with FcεRI engagement; n = 7. **D,** The amount of annexin V–positive pDC (depicted as percentage of annexin $V^+CD123^+BDCA^+$ pDCs on the *y*-axis) of unstimulated *(Ø/white bar)* and FcεRI-activated pDC via IgE and rabbit antihuman IgE *(+/black bar)* after 18 hours of stimulation is shown; n = 8.

FcεRI aggregation on pDCs significantly reduced the intracellular amount of IFN-α and IFN-β detectable in pDCs after CpG-A stimulation (Fig 5, *B*).

DISCUSSION

Although it is generally agreed that pDCs are involved in the elicitation of T_H2-type immune responses, the exact mechanisms underlying this process have not been elucidated so far.[1] Here we show for the first time that $CD123^+BDCA\text{-}2^+$ pDCs in the peripheral blood of patients with AD express enhanced amounts of the trimeric variant of FcεRI, which, similar to myeloid DCs, consists of the IgE-binding α-chain and the γ-chain dimer and is mostly occupied with IgE molecules.[18] In contrast with the FcεRI-bearing peripheral blood DC subtype that was investigated some years ago, $CD123^+BDCA\text{-}2^+$ pDCs shown in this study do not express CD11a, CD11b, or CD14 (data not shown) and therefore represent a distinct FcεRI-bearing pDC subtype.[2,19] Further, we showed that the FcεRI expression on $CD123^+BDCA\text{-}2^+$pDCs is deeply dependent on the atopic state and IgE serum level of the individuals. This observation is of critical immunologic importance because it has been shown recently that the FcεRI surface expression of DCs is contingent on the presence of IgE molecules in the surrounding micromilieu, which obviously forms the basis for the stabilization of the FcεRI complex on the surface of mDCs and—as shown in this study—of $CD123^+BDCA\text{-}2^+$ pDCs.[10,20-22] In the light of these data, the apparent existence of IgE-FcεRI complexes on the surface of pDCs opens several exciting possibilities for the interaction of these cells in T_H2-mediated diseases such as AD. pDCs are capable of taking up allergens via allergen-specific IgE on their cellular surface *in vitro*, which links to a role of these cells in very specific processes leading to allergen internalization, migration of pDCs to the peripheral lymph nodes, and presentation of the processed allergens to allergen specific T cells *in vivo*.[23,24] Here we could show that the preloading of pDCs with allergen specific IgE and recombinant allergens leads to the amplification of T_H2 immune responses of cocultured T cells.[1,25] This leads to the assumption that allergen challenge of pDCs might enhance priming of T_H2 cells and reduce immune responses of the T_H1 type in the periphery, thereby amplifying the outcome of immune reactions of the T_H2 type in patients with AD.[26,27] In this way, pDCs might strengthen both primary and secondary T_H2-dominated allergic immune responses in AD *in vivo*.[28]

Further, FcεRI on the surface of pDCs is functional, and FcεRI aggregation goes along with the downregulation of

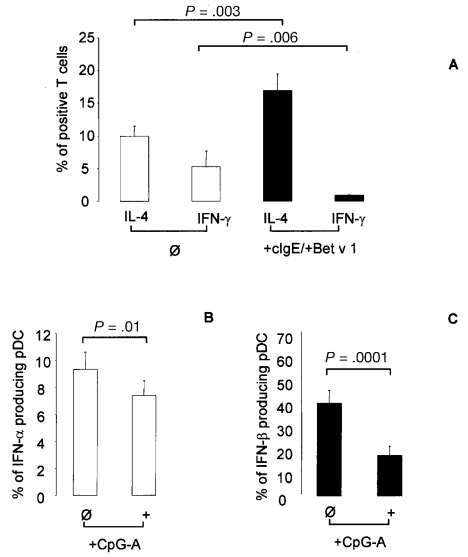

FIG 5. FcεRI stimulation of pDCs reduces their capacity to produce IFN-α and IFN-β and promotes T_H2 immune responses. **A,** The amount of IL-4–producing T cells is enhanced after coculture of autologous naive cells with pDCs preloaded with allergen-specific IgE and recombinant allergen *(black bars)* in comparison with pDCs cocultured with naive T cells in the absence of allergens; n = 12. The percentage of intracellular IFN-α *(white bars)* **(B)** and IFN-β *(black bars)* **(C)** produced in consequence of stimulation with CpG-A of pDCs with *(+)* and without *(Ø)* FcεRI preactivation by mAb against FcεRI (22E7) and goat-antimouse IgG $(Fab')^2$; n = 15 experiments.

costimulatory signals and the expression of MHC molecules. The downregulation of MHC I and MHC II molecules and the costimulatory molecule CD40 after FcεRI engagement might form the basis for a putatively deficient stimulation of distinct T-cell subtypes, leading to the impaired defense against viral infections observable in patients with AD *in vivo*. One possible reason for this phenomenon might be the enhanced production of the immunosuppressive cytokine IL-10 and a putative endogenous feedback of IL-10 on the stimulatory function of pDCs in consequence of FcεRI aggregation, as shown for mDCs in previous studies.[29] Because several studies have confirmed that pDCs are able to promote both T_H1 and T_H2 responses,[30-32] a different maturation stage of pDCs in AD in combination with a distinct regulation of their functional capacities by soluble and cellular factors from their microenvironment might contribute to the functional differences of pDCs in AD evaluated in this study. Further, it is important to notice that although patients with AD display enhanced amounts of pDC in the peripheral blood,[26] the number of pDCs in the skin lesions remains relatively reduced in comparison with other inflammatory skin diseases such allergic contact dermatitis or lupus erythematodes.[33,34] In view of the data appraised here, one might speculate that the lower expression of the skin homing molecules CLA and CD62L on peripheral blood pDCs of patients with AD might lead to a reduced recruitment of pDCs into the skin in patients with AD.[35,36] pDCs have been shown to be crucial for defense against virus infections in the human immune system and are therefore capable of producing high amounts of type I IFNs after stimulation with viral stimuli. Considering the data presented here, the frequent allergen challenge and FcεRI activation on the surface of pDCs in AD might contribute to a reduced capacity of pDCs to produce IFN-α and IFN-β in response to viral infections *in vivo*. One additional mechanism that might lead to an impairment of type I IFN–producing pDCs in the skin of patients with AD might be an enhanced local apoptosis of pDCs induced by IL-10 released in consequence of frequent stimulations of FcεRI-bearing pDCs by allergens together with the increased amount of the T_H2 cytokine IL-4 in the skin lesions. Both of these soluble factors have been shown to influence the viability of pDCs *in vitro*.[37] Moreover, an endogenous downregulatory effect of IL-10 produced by pDCs on the IFN-α–producing and INF-β–producing capacity of pDCs, which has been described in previous studies,[38] might amplify this process. However, the detailed mechanisms underlying the higher susceptibility to viral infections of patients with AD and the consequences arising from the functional differences of pDCs of patients with AD in the context of the development of effective therapeutic strategies remain to be elucidated.

REFERENCES

1. 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.
2. Dzionek A, Inagaki Y, Okawa K, Nagafune J, Rock J, Sohma Y, et al. Plasmacytoid dendritic cells: from specific surface markers to specific cellular functions. Hum Immunol 2002;63:1133-48.
3. Dzionek A, Sohma Y, Nagafune J, Cella M, Colonna M, Facchetti F, et al. BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction. J Exp Med 2001;194:1823-34.
4. Banchereau J, Paczesny S, Blanco P, Bennett L, Pascual V, Fay J, et al. Dendritic cells: controllers of the immune system and a new promise for immunotherapy. Ann N Y Acad Sci 2003;987:180-7.
5. Prussin C, Griffith DT, Boesel KM, Lin H, Foster B, Casale TB. Omalizumab treatment downregulates dendritic cell FcepsilonRI expression. J Allergy Clin Immunol 2003;112:1147-54.
6. Matsuda H, Suda T, Hashizume H, Yokomura K, Asada K, Suzuki K, et al. Alteration of balance between myeloid dendritic cells and plasmacytoid dendritic cells in peripheral blood of patients with asthma. Am J Respir Crit Care Med 2002;166:1050-4.
7. Kinet JP. The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology. Annu Rev Immunol 1999;17:931-72.
8. Bieber T, de la Salle H, Wollenberg A, Hakimi J, Chizzonite R, Ring J, et al. Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (Fc epsilon RI). J Exp Med 1992;175:1285-90.
9. Novak N, Kraft S, Bieber T. Unraveling the mission of FcepsilonRI on antigen-presenting cells. J Allergy Clin Immunol 2003;111:38-44.
10. Novak N, Tepel C, Koch S, Brix K, Bieber T, Kraft S. Evidence for a differential expression of the FcepsilonRIgamma chain in dendritic cells of atopic and nonatopic donors. J Clin Invest 2003;111:1047-56.
11. Maurer D, Ebner C, Reininger B, Petzelbauer P, Fiebiger E, Stingl G. Mechanisms of Fc epsilon RI-IgE-facilitated allergen presentation by dendritic cells. Adv Exp Med Biol 1997;417:175-8.
12. Novak N, Bieber T, Katoh N. Engagement of Fcepsilon RI on human monocytes induces the production of IL-10 and prevents their differentiation in dendritic cells. J Immunol 2001;167:797-804.

13. Von Bubnoff D, Matz H, Frahnert C, Rao ML, Hanau D, de la SH, et al. FcepsilonRI induces the tryptophan degradation pathway involved in regulating T cell responses. J Immunol 2002;169:1810-6.
14. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venerol 1980;92:44.
15. Jurgens M, Wollenberg A, Hanau D, de la Salle H, Bieber T. Activation of human epidermal Langerhans cells by engagement of the high affinity receptor for IgE, Fc epsilon RI. J Immunol 1995;155: 5184-9.
16. Maurer D, Fiebiger E, Reininger B, Ebner C, Petzelbauer P, Shi GP, et al. Fc epsilon receptor I on dendritic cells delivers IgE-bound multivalent antigens into a cathepsin S-dependent pathway of MHC class II presentation. J Immunol 1998;161(6):2731-9.
17. Krug A, Rothenfusser S, Hornung V, Jahrsdorfer B, Blackwell S, Ballas ZK, et al. Identification of CpG oligonucleotide sequences with high induction of IFN-alpha/beta in plasmacytoid dendritic cells. Eur J Immunol 2001;31:2154-63.
18. Novak N, Kraft S, Bieber T. IgE receptors. Curr Opin Immunol 2001;13: 721-6.
19. Maurer D, Fiebiger S, Ebner C, Reininger B, Fischer GF, Wichlas S, et al. Peripheral blood dendritic cells express Fc epsilon RI as a complex composed of Fc epsilon RI alpha- and Fc epsilon RI gamma- chains and can use this receptor for IgE-mediated allergen presentation. J Immunol 1996; 157:607-16.
20. Sihra BS, Kon OM, Grant JA, Kay AB. Expression of high-affinity IgE receptors (Fc epsilon RI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects: relationship to total serum IgE concentrations. J Allergy Clin Immunol 1997;99: 699-706.
21. Saini SS, Klion AD, Holland SM, Hamilton RG, Bochner BS, Macglashan DW Jr. The relationship between serum IgE and surface levels of FcepsilonR on human leukocytes in various diseases: correlation of expression with FcepsilonRI on basophils but not on monocytes or eosinophils. J Allergy Clin Immunol 2000;106:514-20.
22. Borkowski TA, Jouvin MH, Lin SY, Kinet JP. Minimal requirements for IgE-mediated regulation of surface Fc epsilon RI. J Immunol 2001;167: 1290-6.
23. 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.
24. Lambrecht BN. Allergen uptake and presentation by dendritic cells. Curr Opin Allergy Clin Immunol 2001;1:51-9.
25. Charbonnier AS, Hammad H, Gosset P, Stewart GA, Alkan S, Tonnel AB, et al. Der p 1-pulsed myeloid and plasmacytoid dendritic cells from house dust mite-sensitized allergic patients dysregulate the T cell response. J Leukoc Biol 2003;73:91-9.
26. Uchida Y, Kurasawa K, Nakajima H, Nakagawa N, Tanabe E, Sueishi M, et al. Increase of dendritic cells of type 2 (DC2) by altered response to IL-4 in atopic patients. J Allergy Clin Immunol 2001;108:1005-11.
27. Novak N, Valenta R, Bohle B, Laffer S, Haberstok J, Kraft S, et al. FcepsilonRI engagement of Langerhans cell-like dendritic cells and inflammatory dendritic epidermal cell-like dendritic cells induces chemotactic signals and different T-cell phenotypes in vitro. J Allergy Clin Immunol 2004;113:949-57.
28. Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A, et al. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. Nat Med 1999;5:919-23.
29. Nolan KF, Strong V, Soler D, Fairchild PJ, Cobbold SP, Croxton R, et al. IL-10-conditioned dendritic cells, decommissioned for recruitment of adaptive immunity, elicit innate inflammatory gene products in response to danger signals. J Immunol 2004;172:2201-9.
30. Krug A, Veeraswamy R, Pekosz A, Kanagawa O, Unanue ER, Colonna M, et al. Interferon-producing cells fail to induce proliferation of naive T cells but can promote expansion and T helper 1 differentiation of antigen-experienced unpolarized T cells. J Exp Med 2003;197:899-906.
31. Kadowaki N, Antonenko S, Lau JY, Liu YJ. Natural interferon alpha/beta-producing cells link innate and adaptive immunity. J Exp Med 2000;192:219-26.
32. Cella M, Facchetti F, Lanzavecchia A, Colonna M. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. Nat Immunol 2000;1:305-10.
33. Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL. Plasmacytoid dendritic cells (natural interferon-alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions. Am J Pathol 2001;159:237-43.
34. Wollenberg A, Wagner M, Gunther S, Towarowski A, Tuma E, Moderer M, et al. Plasmacytoid dendritic cells: a new cutaneous dendritic cell subset with distinct role in inflammatory skin diseases. J Invest Dermatol 2002;119:1096-102.
35. Bangert C, Friedl J, Stary G, Stingl G, Kopp T. Immunopathologic features of allergic contact dermatitis in humans: participation of plasmacytoid dendritic cells in the pathogenesis of the disease? J Invest Dermatol 2003;121:1409-18.
36. 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;165:4062-8.
37. Wollenberg A, Wetzel S, Burgdorf WH, Haas J. Viral infections in atopic dermatitis: pathogenic aspects and clinical management. J Allergy Clin Immunol 2003;112:667-74.
38. Duramad O, Fearon KL, Chan JH, Kanzler H, Marshall JD, Coffman RL, et al. IL-10 regulates plasmacytoid dendritic cell response to CpG-containing immunostimulatory sequences. Blood 2003;102:4487-92.