A Subset of Human Dendritic Cells Expresses IgA Fc Receptor (CD89), Which Mediates Internalization and Activation Upon Cross-Linking by IgA Complexes

Frédéric Geissmann, Pierre Launay, Benoit Pasquier, Yves Lepelletier, Michelle Leborgne, Agnès Lehuen, Nicole Brousse and Renato C. Monteiro

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Immature dendritic cells (DC) sample Ags within nonlymphoid tissues and acquire exogenous proteins/pathogens via scavenger receptors or Ig FeR such as FcγR and FcεR. IgA is a significant proportion among serum Ig and is the main isotype in mucosas, where DC are numerous. We found that a functional FcεR (CD89) was expressed in situ and in vitro on interstitial-type DC but not on Langerhans cell-type DC. Intertitial-type DC expressed CD89 as a 50- to 75-kDa glycoprotein with a 32-kDa protein core, which was down-regulated upon addition of TGF-β1. DC, FcεR specifically, bound IgA1 and IgA2. Cross-linking of CD89 on DC triggered endocytosis in time-dependent manner. In addition, internalization of polymeric IgA complexes induced the production of IL-10 and DC activation, as reflected by up-regulation of CD86 costimulatory molecules, class II MHC expression, and increased allostimulatory activity. Therefore, interstitial-type DC may use FcεR-mediated Ag sampling in the subepithelium to check tissue integrity while Langerhans cells inside epithelial layers may neglect IgA immune complexes. The Journal of Immunology, 2001, 166: 346--352.

Dendritic cells (DC) are the major human APCs. They have the unique capacity to do the following: 1) sample Ags at the boundary with the "external milieu," 2) migrate toward T cell areas of draining lymph nodes, 3) mature to express costimulatory molecules such as CD86, and 4) trigger immune responses (1). They differentiate from their blood precursors into immature phagocytic DC, which are present in most nonlymphoid tissues, acting as sentinels. One example is Langerhans cells (LC), which are found in the oral, bronchiolar, vaginal, rectal, and cutaneous epithelia (1). Immature DC are efficient in Ag uptake and possess scavenger receptors and selective Ig receptors such as FcγR and FcεR (1–8). The latter contribute to the capacity of DC to process Ags via specific pathways and transfer them to secondary lymphoid organs.

IgA is the most abundant Ig isotype in mucosal tissues and the second isotype in the blood compartment representing one-fifth of IgG levels (9, 10). IgA exists in two subclasses: IgA1 is the predominant isotype in serum in monomeric form and IgA2 is more prevalent in mucosal secretions in dimeric form known as secretary IgA (9, 10). The IgA FcγR (FcεR or CD89) is expressed on human blood neutrophils, eosinophils, and monocyte/macrophages as a 55- to 100-kDa heterogeneously glycosylated protein (11–13). CD89 is a type I transmembrane molecule that is encoded by a single gene located on chromosome 19q13.4 (12, 14). FcεR exists in two known isoforms (a.1 and a.2) differently expressed on blood monocytes and alveolar macrophages (15). CD89 is a receptor for IgA1 and IgA2 Abs that binds more polymeric than monomeric forms (16, 17). Following aggregation of FcεR, IgA immune complexes (IC) can trigger effector cells to perform functions such as endocytosis, phagocytosis, superoxide release, and release of cytokines including TNF-α, IL-6, and Ab-dependent cellular cytotoxicity (14, 18–21).

CD89 is a candidate molecule for Ag uptake by DC, as these cells are particularly numerous at malpighian epithelial (LC-type DC) plus subepithelial (interstitial-type DC) barriers, but it is not known whether human DC bear an IgA receptor. We thus investigated CD89 expression and function on DC and detected functional CD89 molecules on a subset of DC both in vitro and in vivo. We found that CD89 was expressed on interstitial-type DC but not detectable on LC in vivo, and down-regulated by TGF-β1 on Langerhans-type DC in vitro. DC FcεR protein bound IgA1 and IgA2 and was quickly internalized upon aggregation. Cross-linking of polymeric IgA on DC, which mimics the effect of IC, led to IL-10 production and DC activation.

Materials and Methods

Antibodies

The following mouse mAb were used: A59 (IgG1κ) and A77 (IgG1κ), specific for FcεR (22); IV.3 (IgG2b) (American Type Culture Collection, Manassas, VA), specific for FcγRIII; 3G8 (IgG1κ), specific for FcγRIII; and an irrelevant IgG1κ control (clone 7.1 anti-GST protein). The anti-FcεR mAb My43 (IgMκ) was a generous gift from Dr. L. Shen (Dartmouth Medical School, Lebanon, NH). Unconjugated CD68 and HLA-DR,
unconjugated and FITC-labeled CD1a (clone BL6, IgGl) and FITC-labeled HLA-DR were obtained from Immunotech (Marseille, France). PE-labeled anti-FcγR mAb A59 (A59-PE) and CD86 were obtained from Pharmingen (San Diego, CA). Rabbit anti-mouse Ig Abs were raised in rabbits immunized with IgGl (clone A59), F(ab')2, of A59, A77, and IgGlκ, and rabbit anti-mouse IgG fractions were prepared by pepsin digestion (Sigma, St. Louis, MO) and purified on DEAE columns. FITC-conjugated goat Ab specific for mouse (GAM) and rabbit Ig, and HRP-conjugated goat anti-rabbit IgG, were obtained from Southern Biotechnology Associates (Birmingham, AL). IgA myeloma proteins were purified from patients' sera as described (11), and preparations of monomeric and polymeric IgA1x and an IgA2 (>98% pure) were bio- tylated. IgA1x Fab were obtained by digestion with IgGl protease as described (11).

Cell culture

The human monocyte cell line U937 was maintained in RPMI 1640 medium supplemented with 10% FCS, 2 mM glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin (complete medium). In some experiments, cells were cultured for 18 h with 10 μg/ml PMA (Sigma). Monocyte-derived interstitial- and Langerhans cell-type DC were prepared as previously described (23, 24). Briefly, fresh CD14+ monocytes were isolated from healthy volunteers' PBMC obtained by the standard Ficoll-Paque method and immediately separated by negative magnetic depletion using hapten-coated CD3, CD7, CD19, CD34, and anti-IgE Abs (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) and a magnetic cell separator (MACS) according to the manufacturer’s instructions. Cells were cultured in flasks or in 6- or 24-well tissue culture plates (Costar, Cambridge, MA) for 5–7 days in complete medium supplemented with 200 ng/ml GM-CSF and 10 ng/ml IL-4, resulting in their differentiation into CD1a+ interstitial-type DC, or with GM-CSF, IL-4, and 10 ng/ml TGF-β1, resulting in their differentiation into CD1a+ E-cadherin+, CLA+, Langerin+ Lαd+, and Birbeck+ Langerhans cell-type DC.

Flow cytometry

For single- and two-color flow cytometry, 3 × 106 cells were preincubated with 10 μg/ml human polyclonal IgGl to block FcγR (22) in 96-well plates (Becton Dickinson, Mountain View, CA) for 15 min at 4°C in PBS containing 2% FCS, and then incubated for 30 min at 4°C in PBS containing 2% FCS, 0.1% NaN3, and incubated with mAbs at the appropriate concentration (0.1 μg/ml for anti-CD89 mAbs), or with control isotype-matched Ig. After incubation, cells were washed 3 times and incubated with 10 μg/ml human polyclonal IgGl, or with control isotype-matched Ig. After incubation, cells were washed again and incubated for 15 min with streptavidin-PE to label-bound IgGl. For IgGl binding, 106 cells were preincubated in 96-well plates with human IgGl for 15 min at 4°C in PBS containing 2% FCS, and then incubated for 30 min at 4°C with a blocking anti-CD89 mAb (100 μl My43 supernatant) or control Ig Gl hybridoma supernatant, then washed and incubated for 1 h with biotinylated monomeric or polymeric IgGl at 0.1 μg/ml. Cells were washed again and incubated for 15 min with streptavidin-PE to label-bound IgGl. As a negative control, cells were incubated with a biotinylated IgGl Fab (11). Cells were analyzed by flow cytometry using a FACScalibur (Becton Dickinson) and CellQuest software (Becton Dickinson). For IgGl binding, 106 cells were preincubated in 96-well plates with human IgGl for 15 min at 4°C in PBS containing 2% FCS, and then incubated for 30 min at 4°C with a blocking anti-CD89 mAb (100 μl My43 supernatant) or control Ig Gl hybridoma supernatant, then washed and incubated for 1 h with biotinylated monomeric or polymeric IgGl at 0.1 μg/ml. Cells were washed again and incubated for 15 min with streptavidin-PE to label-bound IgGl. As a negative control, cells were incubated with a biotinylated IgGl Fab (11). Cells were analyzed by flow cytometry using a FACScalibur (Becton Dickinson) and CellQuest software (Becton Dickinson).

Cell radiolabeling and immunoprecipitation

Cell surface proteins were iodinated with 1 mCi Na125I (Amerham, Arlington Heights, IL) using the lactoperoxidase method. For FcγR immunoprecipitation, cells (106/ml) were lysed for 30 min at 4°C in PBS containing 0.5% Nonidet P-40 (Aldrich Chemical, Milwaukee WI), 0.02% sodium azide, 1% aprotinin, 1 mM diisopropylfluorophosphate, 5 mM iodoacetamide, and 1 mM PMSF (11). After centrifugation at 14,000 g for 30 min to remove insoluble material, cleared lysates were immunodepleted for FcγR by using human IgGl, 32.2, 3G8, and IV.3 mAb, and precipitated with test mAb as previously described (15, 22). Bound material was treated or not treated with N-glycosidase F (Oxford GlycoSystems, Abingdon, U.K.) and samples were prepared for SDS-PAGE.

RT-PCR analysis

Total RNA was extracted by the acid-phenol procedure and cDNA synthesis and PCR were performed as described previously (15).

Immunohistochemistry

Normal skin and gingival samples were obtained from the tissue bank of the pathology department of Hôpital Necker-Enfants Malades. Serial cryostat sections were prepared and incubated with anti-CD1a (BL1), HLA-DR (B8.12.2), and CD68 (KiM7) from Immunotech, and anti-CD89 (A59, A77) mouse primary Abs, followed by a goat anti-mouse alkaline phosphatase (AP)-conjugated Ab. Double labeling was performed using peroxidase and AP-anti-AP protocols as described elsewhere (25). Fast Blue and 3-aminio-9-ethylcarbazole (both from Sigma) were used as substrates for AP and peroxidase, respectively.

Confluent microscopy and endocytosis

Cells were allowed to adhere to glass slides coated with 50 μg/ml poly-L-lysine (Sigma), and consecutively incubated on ice with human polyclonal IgGl, mAb A77, and F(ab')2; GAM-FITC (Southern Biotechnology Associates) in PBS containing 2% FCS. After washing, cells were incubated in complete medium for various times at 37°C or kept on ice. Slides were washed, fixed in 4% paraformaldehyde in PBS, and quenched with 0.1 M glycine. Cells were then incubated for 5 min with 2 μg/ml wheat germ agglutinin (WGA) conjugated to Texas Red and washed again. Mounted slides were examined with a confocal laser microscope system (LSM 510 Carl Zeiss; Zeiss, Jena, Germany).

Activation of DC

DC cultured for 6 days were gently washed and preincubated with blocking anti-CD89 mAb (My43) or irrelevant IgGl (clone D6), and then incubated on ice for 60 min with biotinylated IgGl. IgGl, anti-CD4 mAb, or RPMI 1640–2% human AB serum. Cells were gently washed again and incubated on ice for 20 min with streptavidin-AP to cross-link surface receptors or RPMI 1640–2% human AB serum as negative control. After a final wash, cells were cultured at 5 × 106/ml in complete medium supplemented with 200 ng/ml GM-CSF and 10 ng/ml IL-4. At various times, cells were harvested and their viability, as well as CD86 and DR expression, were assessed by trypan blue exclusion and flow cytometry, respectively.

Allogeneic T cell proliferation

DC cultured for 40 h as described above after incubation with biotinylated IgGl A, or RPMI 1640–2% human AB serum and with or without streptavidin-AP to cross-link surface receptors were collected, washed three times, resuspended in RPMI 1640 with 10% human AB serum and added in triplicate at various concentrations to 104 allogeneic T cells/well in 96-well tissue culture plates (Falcon; Becton Dickinson). T cells were isolated by the standard Ficoll-Paque method followed by magnetic depletion of non-T cells (MACS; Miltenyi Biotec). [3H]Thymidine (Amersham Life Sciences, Buckinghamshire, U.K.) incorporation was measured in newly synthesized DNA over 18 h, using pulses initiated at day 5 of the culture with 1 mCi well of [3H]thymidine. Cells were then harvested with a 96-well harvester (Pharmacia, St. Quentin, France), collected on glass-fiber filter (Pharmacia), and the incorporation of thymidine was measured with a β-plate microsountiation counter (LK, Pharmacia).

Measurement of IL-10 production by ELISA

Culture supernatants were harvested after 24 and 48 h of culture, and centrifuged twice at 200 × g and 15,000 × g to remove cells and debris. Supernatants were stored at −70°C until cytokine measurements. ELISA was performed in duplicate and according to the manufacturer’s instructions using human quantikine IL-10 (R&D Systems, Minneapolis, MN) and rHL-10 provided by the manufacturer at 500–7.8 pg/ml. The sensitivity of IL-10 detection was thus 7.8 pg/ml.

Results

CD89 detection on dermal macrophage/DC but not on epidermal LC

The cellular distribution of CD89 in normal skin and mucosa was investigated by means of immunohistochemistry. Frozen skin and gingival tissue sections were labeled with anti-CD1a, CD89, DR, or CD68 Abs. An example of CD1a labeling on LC is shown in Fig. 1A. An anti-CD89 Ab (A59) did not stain CD1a+ epidermal LC but labeled dermal cells on serial sections (Fig. 1B, magnification in C). Another anti-CD89 Ab (A77) yielded an identical labeling pattern (not shown). Double labeling with anti-DR (in blue) and anti-CD89 (in brown) showed two populations of CD89+ cells in the dermis, one strongly coexpressing DR (and thus stained bluish red) and the other DR− (Fig. 1D). Both dermal populations were CD68+ (not shown). Therefore, while CD89 was
undetectable on LC, dermal macrophages/DC were CD89⁻. Similar labeling patterns were observed in gingival mucosa (Fig. 1E). Absence of CD89 labeling on LC was not likely to be due to receptor site occupancy, because there was no LC labeling for IgA on samples from three individuals (data not shown).

**CD89 expression on monocyte-derived DC in vitro**

To investigate the regulation of CD89 expression on DC, we used freshly isolated CD14⁺ blood monocytes differentiated into DC in the presence of GM-CSF and IL-4 (2). These cells share many features with interstitial DC and particularly with dermal DC, which in the presence of TGF-β₁, further differentiate toward the LC phenotype (2, 23–25). CD14⁺ CD1a⁻ peripheral blood monocytes, CD1a⁺ E-cadherin⁻ Langerin⁻ monocyte-derived interstitial-type DC and CD1a⁺ E-cadherin⁺ Langerin⁺ monocyte-derived Langerhans cell-type DC were thus studied for CD89 expression by means of immunofluorescence, SDS-PAGE, and RT-PCR.

Flow cytometry experiments revealed that CD89 expression on monocyte-derived interstitial-type DC (CD14low/CD1a¹ E-cadherin²) expressed CD89 levels that were significant but lower than those expressed by monocytes (Fig. 2 and Table I). CD89 expression was barely detectable on LC-type cells (CD14² CD1a¹ E-cadherin¹), with about 3-fold lower levels than DC-type cells calculated from five experiments (Fig. 2 and Table I).

Therefore, to characterize the molecular nature of CD89 on DC, we examined the biochemical characteristics of CD89 on both types of DC. Iodinated cell surface protein was immunoprecipitated with an anti-FcαR mAb (A77) F(ab')² (to avoid interaction with FcγR) from a monocytic cell line (U937), monocyte-derived DC, and monocyte-derived LC (Fig. 3A). DC CD89 appeared as 50- to 75-kDa heterogeneously glycosylated molecules. When immunoprecipitated molecules were digested with N-glycosidase F, FcαR protein was resolved as a major band of 32 kDa, indicating that monocyte-derived interstitial-type DC and LC-type DC expressed the same backbone as the CD89 from monocytic cell line U937 (Fig. 3A). SDS-PAGE also revealed a strong down-regulation of FcαR on the cell surface of LC (Fig. 3A), despite the slightly higher CD1a expression on LC-type as compared with interstitial-type DC (Fig. 3B). RT-PCR analysis was then performed and showed that monocyte-derived interstitial DC and Langerhans cell-type DC expressed the CD89 full-length transcript of 896 bp (a1 transcript) as observed on monocytes and U937 cells (data not shown) and as previously described (19). These results indicate that monocyte-derived interstitial-type DC and LC-type DC expressed the a1 isoform, which is the main isoform expressed on blood monocytes, neutrophils, and eosinophils (11, 13, 15).

**CD89 on DC binds human IgA**

Monocyte-derived interstitial- and LC-type DC, and blood monocytes (used as controls) were then studied for their ability specifically to bind polymeric and monomeric IgA. To assess binding specificity, cells were preincubated with a blocking anti-CD89 (My43) Ab or with irrelevant IgM. Monocytes and monocyte-derived DC bound polymeric IgA (Fig. 4) and to a lesser extent monomeric IgA (data not shown). Both cell types also bound to both the DR- (brown labeling) and DR⁺ dermal cell populations (see arrows indicating double-stained bluish red cells). E. Double labeling with anti-CD1a (red) and anti-CD89 (blue) on healthy gingiva. As observed in the skin, CD89 labeling (+) is restricted to dermal cells and CD1a (→) to the epithelium.
myeloma IgA2 and secretory IgA (data not shown). Preincubation of monocyte-derived interstitial-type DC with My43 Ab inhibited polymeric IgA1 binding by 60–80% (Fig. 4 and Table II). In contrast, monocyte-derived LC exhibited very weak polymeric IgA1 binding, which correlates with weak CD89 expression (Fig. 4 vs Figs. 2 and 3). However, the dim IgA binding was inhibited by My43 mAb (Fig. 4 and Table II).

CD89-mediated IgA internalization up-regulates class II and CD86 expression on interstitial-type DC

To examine the functional capacity of CD89 on DC, we investigated whether internalization occurred after FcεR cross-linking on interstitial-type DC using confocal microscopic examination after cell incubation for various times at 37°C. CD89 cross-linking was performed with either anti-CD89 plus anti-mouse Ab (both in F(ab\(^9\))\(^2\) form) (Fig. 5) or polymeric IgA (not shown), on poly-L-lysine-coated slides. As shown in Fig. 5A, CD89 was only detected at the plasma membrane, colocalizing with WGA labeling on cells kept at 4°C, whereas the majority of CD89 labeling was intracellular after 15 min of incubation at 37°C (Fig. 5B). In contrast, after 1 h at 37°C, cross-linked CD89 molecules were found at the periphery, near the plasma membrane (Fig. 5C), pointing to receptor recycling.

To determine whether, as described for mouse IgG receptor (26), CD89 triggering activates interstitial-type DC, we examined the capacity of IgA complexes to increase costimulatory molecule (CD86) and MHC class II expression in comparison with TNF-α in culture. Cells were incubated at 4°C with TNF-α, biotinylated

![Image](https://example.com/image1)

**FIGURE 2.** Regulated expression of CD89 on monocyte-derived interstitial- and LC-type DC. Using the mAb A77, CD14\(^+\) CD1a\(^-\) monocytes stained strongly positive for CD89 (middle panels), while CD89 labeling was decreased on monocyte-derived E-cadherin\(^-\) DC (bottom panels) and further down-regulated on TGF-β1-treated E-cadherin\(^-\) monocyte-derived LC (bottom right panel). The numbers indicate the mean fluorescence intensity (MFI) for CD89 labelings.

![Image](https://example.com/image2)

**FIGURE 3.** Biochemical characterization of CD89 on monocyte-derived interstitial- and LC-type DC. A. Iodinated cell surface proteins from a monocytic cell line (U937), monocyte-derived DC, and monocyte-derived LC were immunoprecipitated by an anti-FcεR A77 F(ab\(^9\))\(^2\). FcεR molecules with an M\(_\text{r}\) of 50–75 kDa were specifically precipitated and resolved into a major band of 32 kDa (isoform a1) when digested with N-glycosidase F (N-gly). B. Iodinated cell surface proteins from the same samples were immunoprecipitated by an anti-CD1a mAb (BL6).

![Image](https://example.com/image3)

**FIGURE 4.** IgA binding on monocyte-derived interstitial- and LC-type DC. Monocytes (top panel), monocyte-derived DC (middle panel), and monocyte-derived LC (bottom panel) bound polymeric IgA1k (shaded area) with variable efficiency. Biotinylated IgA1 Fab was used as a negative control (dashed line). Preincubation with the blocking anti-CD89 mAb My43 inhibited IgA binding (solid line).

<table>
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<th>Expt. No.</th>
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<th>Mo-DC</th>
<th>Mo-LC</th>
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<td>100(^b)</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
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<td>–</td>
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<tr>
<td>Mean ± SEM</td>
<td>18.2 ± 4</td>
<td>6.4 ± 2*</td>
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</table>

\(^a\) MFI of A77 labeling (FACS analysis) on monocytes, U937 cells, and monocyte-derived DC and LC.

\(^b\) PBMC.

\(^c\) U937 cell line.

\(^*\) p < 0.05 Student’s t test.

![Image](https://example.com/image4)

**Table I.** CD89 expression on monocyte-derived interstitial-type (Mo-DC) and Langerhans cell-type (Mo-LC) DC using anti-CD89 mAb

![Image](https://example.com/image5)
polymeric IgA (0.1–0.01 mg/ml), biotinylated IgG at the same concentration or biotinylated anti-CD4 mAb (negative control), and then with or without a cross-linker, streptavidin coupled to a carrier molecule (AP). Cells were then washed and cultured for various times. As shown in Fig. 6A, while IgA and IgG alone (c and e) failed to activate DC, cross-linking of IgA and IgG (d and f) induced CD86 expression on ~40% of cells. As a positive control, similar activation was observed after cell exposure to 10 ng/ml TNF-α (Fig. 6A, a). In contrast, CD4 cross-linking under the same conditions did not significantly activate DC (Fig. 6A, b). It is noteworthy that DC activation through IgA complexes was specific to FcαR as the preincubation of cells with a blocking anti-CD89 mAb (My43) inhibited their activation by IgA but not by IgG complexes (Fig. 6A, dashed histograms in d and f). Therefore, we performed time-course experiments to analyze by flow cytometry the percentage of double-positive cells expressing high levels of MHC class II (DR) and CD86 molecules in culture (Fig. 6B). IgA complexes induced maximal activation after 2 days of culture in a manner comparable to TNF-α. This was still observed with 0.01 mg/ml of cross-linked IgA (Fig. 6B). This IgA-mediated activation was specific for FcαR as it was inhibited by My43 mAb and was not observed for anti-CD4 cross-linking (Fig. 6B). IgA complexes did not increase costimulatory molecule (CD86) and MHC class II expression on LC-type cells (data not shown). This is in accordance with the very weak expression of CD89 and binding of IgA on these cells (Figs. 1–4).

CD89 triggering on DC results in allogeneic lymphocyte activation and in IL-10 secretion

To examine whether CD89-mediated phenotypic activation of interstitial-type DC was associated with their function, we focused on their capacity to trigger allogeneic lymphocyte activation and to produce IL-10. The results showed that preincubation of DC with IgA complexes significantly increased the proliferation of allogeneic T-lymphocytes at low stimulator/effector ratio (250–1000 DC for 10^5 T cells; Fig. 7A). These effects required the cross-linking of CD89, because IgA alone did not have any effect, and was specific to CD89 because it was abrogated by preincubating cells with anti-CD89 My43 mAb (Fig. 7A). Binding of IgA complexes may thus contribute to the functional maturation of immature interstitial-type DC.

Interstitial-type DC has been shown to produce IL-10 upon in vitro stimulation with TNF-α and IL-1 (26). Therefore, we examined the ability of interstitial-type DC to produce IL-10 after stimulation via CD89 by IgA complexes. Binding of cross-linked IgA induced the production of IL-10 at comparable level to that observed with stimulation by TNF-α and IL-1β, while TNF-α alone failed to induce significant IL-10 production (Fig. 7B). This effect was abrogated by preincubation with My43 and required receptor aggregation as IgA alone failed to induce IL-10 release (Fig. 7B).

### Table II. Specificity of IgA binding to monocyte-derived interstitial-type (Mo-DC) and Langerhans cell-type (Mo-LC) DC

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<tr>
<th>Expt. No.</th>
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<th>Monocytes</th>
<th>Mo-DC</th>
<th>Mo-LC</th>
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<td>3</td>
<td>-</td>
<td>ND</td>
<td>22 (71)</td>
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* Inhibition of IgA1k binding by My43 on cells was performed as described in Fig. 4. The percentage of inhibition was calculated as follows: 100 × (MFI IgA – MFI IgA + My43)/(MFI IgA – MFI control).

* PBMC.

* U937 cell line.

**FIGURE 5.** CD89 on monocyte-derived DC is internalized after cross-linking. Cells were examined by confocal laser microscopy. A, CD89 labeling colocalized with WGA-Texas Red labeling on monocyte-derived DC incubated on ice with mAb A77 F(ab')2 and cross-linked with F(ab')2, GAM-FITC. B, After 15 min at 37°C, CD89 labeling (A77) was intracellular. C, After 60 min at 37°C, CD89 staining (A77) was detected near the periphery.
Discussion

We report here the expression, regulation, and function of the IgA FcR, the CD89, on a subset of human DC in vivo and in vitro. In vivo, interstitial DC/macrophages but not LC expressed CD89. Indeed, CD89 expression was not detectable on LC by immunohistochemistry with two anti-CD89 mAbs. Functional studies could not be performed on sorted interstitial DC because these cells cannot be efficiently isolated. Therefore, we took advantage of an in vitro model of DC differentiation from blood monocytes.

Further differentiation toward LC (23–24). Using this model, we also observed that interstitial-type DC expressed heterogeneously glycosylated a1 isoform of the CD89 that was functional, whereas CD89 expression by Langerhans cell-type DC was weak. The unique cytokine microenvironment in the epithelium plays an important role in regulating the DC phenotype. We and others have shown that TGF-β1, which is produced by keratinocytes in vivo (27), is activated in a tissue-specific manner, and is responsible for the unique phenotype of LC (23, 24, 28). In the present study, CD89 was detected in vivo on dermal cells, but not on LC. Monocyte-derived interstitial-type DC (cultured in the absence of TGF-β1) express CD89 albeit at lower levels than monocytes, while CD89 expression appears to be further down-regulated in monocyte-derived LC-type DC that are obtained by addition of TGF-β1. Furthermore, TGF-β1 has been shown to down-regulate IgA Fe-receptor (CD89) expression on human monocytes (29). Therefore, it is likely that TGF-β1 is responsible for the down-regulation of CD89 on LC in human skin and gingival mucosa. The lack of CD89 labeling on LC in situ does not rule out weak expression. Our results thus point to developmental regulation of CD89 expression on DC, and we were concerned with the potential physiological significance of this observation. We show that CD89 is a selective Ag-binding and Ag-uptake receptor that permitted internalization of its ligand by immature DC, triggered the expression of an in vitro model of DC differentiation from blood monocytes.

FIGURE 6. CD89 cross-linking induces CD86 and DR up-regulation on monocyte-derived DC. A, Cells were analyzed by flow cytometry for CD86 expression after 48 h of culture upon exposure to RPMI 1640–2% human AB serum alone (open histograms), TNF-α (a, filled histogram), biotinylated anti-CD4 Ab cross-linked with streptavidin-AP (b, filled histogram), 0.1 mg/ml biotinylated IgA1κ (c, filled histogram), biotinylated IgA1κ cross-linked with streptavidin-AP (d, filled histogram), 0.1 mg/ml biotinylated IgG (e, filled histogram), and biotinylated IgG cross-linked with streptavidin-AP (f, filled histogram). IgA1κ and IgG alone (c and e) did not activate DC, while CD86 expression was induced on 40% of cells upon cross-linking (d and f), an effect similar to that observed after exposure to 10 ng/ml TNF-α (a). CD4 cross-linking in the same conditions did not activate DC (b), and preincubation of DC with My43 mAb inhibited DC activation by IgA1κ but not by IgG (d and f, dashed open histograms). Data are from one experiment representative of three performed with cells from different donors. B, The percentages of CD86 high and DR high DC were determined by flow cytometry after 24 and 48 h of culture with 10 ng/ml TNF-α (a) or after stimulation (see Materials and Methods) with PBS ( ), biotinylated anti-CD4 Ab cross-linked with streptavidin-AP (Δ), 0.1 mg/ml biotinylated IgA1κ (＊), 0.1 mg/ml biotinylated IgA1κ cross-linked with streptavidin-AP (●), 0.01 mg/ml biotinylated IgA1κ cross-linked with streptavidin-AP (○), or blocking anti-CD89 Ab My43 (●). Data are from the same experiment as in Fig. 5A (representative of three experiments performed with cells from different donors).

FIGURE 7. IgA complexes induced increased allostimulatory activity and IL-10 production by monocyte-derived DC. A, CD89 cross-linking increases DC allostimulatory activity. Cells were cultured for 40 h with medium alone (□), 0.1 mg/ml biotinylated IgA1κ (●), or biotinylated IgA1κ cross-linked with streptavidin-AP without (■) or with preincubation with the blocking anti-CD89 Ab My43 (□). Cells were then cultured for 5 days with allogeneic T cells, and [3H]thymidine incorporation was measured over 18 h. B, FcRmediated release of IL-10 after multivalent cross-linking by IgA complexes. DC were stimulated for 48 h with TNF-α and IL-1, TNF-α, biotinylated IgA1κ, biotinylated IgA1κ cross-linked with streptavidin-AP in the presence of a mouse irrelevant IgM, or with blocking anti-CD89 mAb (My43). Culture supernatants were collected and assayed for IL-10 using specific ELISA.
of the costimulatory molecule CD86 and of MHC class II molecules at the plasma membrane, increased their allostimulatory activity and triggered IL-10 production. IgA plays a major role in preventing pathogen adhesion to the mucosa, and pathogen-IgA is washed out by mucosal secretion. However, one can expect that following mucosal lesions IgA IC could play a role in Ag sampling and presentation by DC in vivo, as well as in DC activation, depending on the site of Ag entry. Indeed, cross-linking of IgA bound to its receptor on interstitial-type DC was the up-regulation of the costimulatory molecule CD86 and membrane MHC class II Ags. Therefore, CD89 triggering by polymeric IgA cross-linking of IgA bound to its receptor on DC was the up-regulation of IgA IC in interstitial tissues or blood may thus result from either breakdown of the epithelial barrier or a blood-borne infection. Our results suggest that, in these circumstances, IgA IC may lead to Ag internalization by interstitial DC, followed by their activation and subsequent Ag presentation to T cells. In contrast, because CD89 expression is down-regulated by TGF-β1 and undetectable by immunohistochemical methods on human epithelial LC in situ, our results suggest that LC may neglect IgA IC within the epithelium in the absence of a breakdown of the epithelial barrier.

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References