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Engagement of FcεRI on Human Monocytes Induces the Production of IL-10 and Prevents Their Differentiation in Dendritic Cells[1]

Natalija Novak,* Thomas Bieber,[2]* and Norito Katoh*†

The local cytokine environment and the presence of stimulatory signals determine whether circulating monocytes will finally acquire characteristics of dendritic cells (DCs) or macrophages. Because FcεRI expressed on professional APCs, e.g., monocytes and DCs, has been suggested to play a key role in the pathophysiology of atopic diseases, we evaluated the effect of receptor ligation on the generation of monocyte-derived DCs (MoDCs). Aggregation of FcεRI at the initiation of the IL-4-GM-CSF-driven differentiation resulted in the emergence of macrophage-like cells with a strong expression of the mannose receptor and a low level of CD1a and the DC-specific markers CD83 and the actin-bundling protein (p55). These cells sustained the ability to take up FITC-labeled *Escherichia coli* by phagocytosis and were significantly less efficient in stimulating purified allogeneic T cells. In addition, receptor ligation of FcεRI at the beginning of the culture prevented the generation of MoDCs, mainly due to a dramatic increase in the IL-10 production. These results suggest that FcεRI aggregation prevents the generation of CD1a$^+$ MoDCs and imply a novel pivotal function of this receptor in modulating the differentiation of monocytes. ***The Journal of Immunology,*** **2001, 167: 797–804.**

It is well accepted that the lack of appropriate exogenous stimuli leads monocytes to undergo apoptosis, whereas, under the influence of distinct signals such as cytokines, these cells differentiate into either macrophages or dendritic cells (DCs)[3] (1–5). Although exhibiting low stimulatory capacity toward resting T cells, macrophages demonstrate a potent capacity of phagocytosis and production of proinflammatory or anti-inflammatory cytokines that potentially regulate inflammatory reactions. In contrast, DCs display low phagocytic activity but an extraordinary capacity for initiating a primary and secondary T cell response and are regarded as the most efficient professional APCs (6). This fundamental functional divergence may be of crucial importance for the putative regulatory role of monocyte-derived DCs (MoDCs) or macrophages in tissues and, consequently, for the outcome of the local inflammatory response.

It has been shown that DCs can be generated in vitro from peripheral blood monocytes by the addition of GM-CSF and IL-4 to the cell culture (4, 5). Interestingly, Pastore et al. demonstrated that supernatants of keratinocytes from lesional skin of atopic dermatitis (AD) that were shown to overproduce GM-CSF, together with exogenous IL-4, could drive phenotypic and functional differentiation of monocytes into DCs (7). This finding could explain why DCs are found in increased numbers in lesional skin of AD, whereas IL-4-producing Th2 cells have been reported to be dominant mainly in acute/initial lesions (8, 9). Thus, DCs may play a major role not only in the primary immune responses but also in allergic reactions (10). In contrast, macrophages are the predominant inflammatory cells in chronic AD lesions (11). These observations suggest that, either in the peripheral blood or in the tissue, monocytes are subjected to a complex network of regulatory signals that direct their differentiation toward either macrophages or DCs (12).

Besides the effector cells of anaphylaxis, FcεRI is also expressed on a variety of APCs including circulating monocytes and DCs and, on the other hand, tissue DCs such as epidermal Langerhans cells and related inflammatory dendritic epidermal cells (13–17). Among the putative roles of this receptor on APCs, it has been shown that FcεRI mediates efficient IgE-dependent allergen uptake and subsequent presentation to T cells (14, 15, 18, 19). Very recently, we have reported that ligation of FcεRI on monocytes protects them from Fas/Fas ligand-induced apoptosis (20), strongly suggesting that this structure also contributes to other mechanisms involved in the outcome of APC differentiation.

In this report, we examined the effect of FcεRI-ligation on the GM-CSF- and IL-4-driven differentiation of monocytes from atopic and nonatopic donors into MoDCs. We demonstrate that 1) in monocytes from atopic donors, cross-linking of FcεRI at the initiation of culture prevents the generation of CD1a$^+$ MoDCs and induces the generation of macrophage-like cells; 2) cross-linking of FcεRI leads to a substantial increase in the secretion of proinflammatory cytokines and chemokines; and 3) this phenomenon results from the receptor-induced secretion of IL-10.

Materials and Methods

Reagents

Rabbit anti-human IgE, anti-human mAb CD23 (MHM6; IgG1) and anti-CD91 (A2 Mrα; IgG1) were purchased from Dako (Glostrup, Denmark). Monomeric human myeloma IgE (hIgE) was obtained from Calbiochem (San Diego, CA) and was filtered for exclusion of the materials with molecular mass of >300 kDa (Ultrafree-MC Filter Unit; Millipore, Bedford, MA). Anti-human FcεRI-α-chain mAb 22E7 was a kind gift from Dr. J.

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[3] Abbreviations used in this paper: DC, dendritic cell; AD, atopic dermatitis; hIgE, monomeric human myeloma IgE; MoDC, monocyte-derived DC; GaM-IgG, F(ab′)$_2$ goat-anti-mouse IgG; MCP, monocyte chemoattractant protein; MFI, mean fluorescence intensity.

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Kochan (Hoffman-LaRoche, Nutley, NJ). PE-labeled mAbs against CD14, CD80, CD86, HLA-DR, IL-1β, IL-1Rα, and their isotype-matched control Abs were obtained from BD Biosciences (Mountain View, CA). The hybridoma cell line that produces mAb W6/32 against MHC class I (21) was kindly provided by Dr. N. Koch (Institute of Immunbiology, University of Bonn, Bonn, Germany). Anti-CD40 (VCD40.4; IgG1), anti-CD68 (Y1/82A; mIgG2b), anti-CD83 (HB15A; mIgG2b), anti-human mannose receptor (mIgG1), and the neutralizing mAbs against TNF-α, IL-10, IL-6, M-CSF, and their isotype-matched controls were obtained from R&D Systems (Minneapolis, MN). PE-labeled mAbs against TNF-α, IL-8, macrophage inflammatory protein-1α, macrophage chemoattractant protein (MCP)-1, MCP-3, and their isotype-matched controls were obtained from BD PharMingen (Hamburg, Germany). The anti-p55-specific mAb against the DC-specific actin-bundling protein (K2; IgG1) was a kind gift from Dr. E. Langhoff (Massachusetts General Hospital, Boston, MA). $F(ab')_2$ (2) goat-anti mouse IgG (GaM-IgG) was obtained from Jackson ImmunoResearch Laboratories (West Grove, PA). FITC-labeled anti-CD1a (OKT6) was obtained from Ortho Diagnostics (Raritan, NJ), and PE-labeled anti-CD64 was obtained from Serotec (Oxford, U.K.). Anti-IL-1β mAb was from obtained from R&D Systems. All other reagents were obtained from Sigma (St. Louis, MO) unless otherwise indicated.

Preparations and cultures

Whole blood was obtained after informed consent from normal, nonatopic healthy volunteers ($n = 18$) and atopic patients with AD ($n = 28$) according to the criteria of Hanifin and Rajka (22). Blood samples were obtained in accordance with the local ethics committee.

Monocytes were isolated from peripheral blood with a modified density gradient protocol after using Nycoprep (Nycomed, Oslo, Norway). Briefly, RBCs were separated from plasma by sedimentation from whole EDTA blood with 1/10 (w/v) 6% dextran 500 in 0.9% NaCl. Plasma was gently layered over Nycoprep and centrifuged for 20 min at $600 \times g$. After separation, the interphase was collected and washed four times with 0.9% NaCl plus 0.13% EDTA and 1% BSA. Then CD14 expression was assessed by flow cytometry. MoDCs were generated as described elsewhere (4) by culturing monocytes for 6 days in medium containing 500 IU/ml human recombinant GM-CSF (Leucomax; Novartis, Basel, Switzerland) and 500 IU/ml human recombinant IL-4 (Life Technologies, Rockville, MD) in 24-well plastic plates. On day 2, 500 IU/ml IL-4 was added into the medium, and the cultures were fed with fresh medium containing 500 IU/ml GM-CSF and 500 IU/ml IL-4 on day 4. Final maturation was obtained by culturing in the presence of IL-1β (10 ng/ml; R&D Systems), IL-6 (1000 U/ml; R&D Systems), and TNF-α (10 ng/ml; R&D Systems).

For the isolation of peripheral blood DCs, PBMC were isolated from heparinized whole blood of normal healthy donors by standard gradient centrifugation with Lymphoprep (Nycomed). PBMC were harvested from the interface, washed twice, and resuspended in PBS supplemented with 5 M EDTA and 0.5% human serum albumin. DCs were obtained directly from PBMC by depletion of monocytes, NK cells, B cells, and T cells by high gradient magnetic sorting using the autoMACS technique (Miltenyi Biotec, Bergisch Gladbach, Germany). mAbs against CD14, CD16, CD56, CD19, and CD3 (Miltenyi Biotec) were used. The negatively selected cells were subsequently incubated with anti-CD4-conjugated magnetic beads, and the positively selected cells were collected.

Basophils were obtained from PBMC by depletion of monocytes, NK cells, DCs, early erythroid cells, platelets, neutrophils, eosinophils, and T cells. A mixture of CD3, CD7, CD14, CD15, CD16, CD36, CD45RA, and anti-HLA-DR Abs (Miltenyi Biotec) was used for the depletion on non-basophils using an indirect magnetic labeling system for the isolation of untouched basophils and the autoMACS technique.

All plastic ware and culture reagents used were tested for the presence of endotoxin with the *Limulus* amebocyte lysate E-Toxate multiple test (Sigma). Endotoxin levels were, in all cases, <10 pg/ml.

Receptor ligation

Cross-linking of FcεRI was achieved as previously described (23). Briefly, the cells were harvested and washed with culture medium twice and then incubated for 1 h with 1 μg/ml hIgE or 10 μg/ml anti-FcεRI mAb $F(ab')_2$ 22E7 or isotype-matched control mIgG1 for 1 h at 37°C. After washing with culture medium, 20 μg/ml anti-human IgE or GaM-IgG was added either for 1 h or for the duration of the whole culture.

To exclude that soluble factors released from other FcεRI-bearing cells of the peripheral blood (i.e., basophils or peripheral blood DCs) affected our culture conditions, experiments were done with cell fractions in which these cell types had been depleted from the monocyte preparation. As a control, basophils and peripheral blood DCs were positively selected by magnetic labeling, and cross-linking experiments were done with these cell types as described above. Then IL-10 production was measured as described below.

Flow cytometric analysis

Immunolabeling for phenotyping was performed as reported previously (17). Intracellular staining for cytokines and chemokines was conducted according to the manufacturer's instructions (BD PharMingen). Briefly, cells cultured in the presence of 1 μM/ml brefeldin A were fixed with 4% paraformaldehyde for 10 min, washed in PBS supplemented with 1% FCS and 0.1% sodium azide, and incubated for 30 min with 1 μg/ml FITC-conjugated CD1a. Then the cells were washed twice with PBS containing 1% FCS, 0.1% sodium azide, and 0.1% saponin. The cells were incubated with PE-labeled mAbs against cytokines or chemokines. After washing with PBS, cells were analyzed on a FACSCalibur (BD Biosciences) as described in detail elsewhere (17). As a control, cells were also stained with corresponding PE-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 the isotype control, and relative fluorescence intensity was calculated from mean fluorescence intensity (MFI) as follows: relative fluorescence intensity = (MFI of cytokine/chemokine − MFI of isotype control)/MFI of isotype control.

Assessment of the phagocytotic activity

The phagocytic activity was tested using the Phagotest from Opregen-Pharma, (Heidelberg, Germany) and was performed according to the manufacturer's protocol. Briefly, cultured cells were incubated with FITC-labeled opsonized bacteria (*Escherichia coli*) at 37°C or 0°C as negative control. Then cold quenching solution was added to suppress fluorescence of the bacteria attached to the external cell wall, and cells were washed twice to remove the supernatant. The percentage of cells having performed phagocytosis was analyzed by flow cytometry.

Proliferation assay

MLRs were conducted in 96-well flat-bottom microtiter plates by adding different numbers of irradiated (3000 rad) cells (ratio of stimulator: responder cells, 1:10, 1:100, 1:1000) to 2×10^5 allogeneic T cells purified from PBMC as described previously (24). After 4 days at 37°C, cell proliferation was assessed by uptake of [^{3}H]thymidine (1.25 μCi/well added for 16 h; Amersham, Little Chalfont, U.K.).

Detection of cytokines

For ELISA, supernatants were stored at −70°C until cytokine measurements. Quantification of IL-10 released from monocytes in the supernatants was conducted in triplicate using a human IL-10 ELISA kit (Genzyme, Cambridge, MA) according to the manufacturer's instructions. Sensitivity of IL-10 detection was 5 pg/ml.

For the ELISPOT assay, the anti-human IL-10 Ab (9D7) was diluted to a concentration of 15 μg/ml in sterile filtered PBS, and 100 μl/well was added to nitrocellulose plates (Millipore, Bedford, MA). Plates were incubated overnight at 4°C, and thereafter unbound Abs were washed away by six successive washings with filtered PBS. Then plates were incubated for 1 h with PBS and 20% FCS (Sigma) to block any unspecific binding sites. A total of 100 μl of cells suspensions was added to each well, and plates were incubated 18 h at 37°C. Thereafter, the cells were washed away and 100 μl biotinylated IL-10 mAb (12G8), and 1 μg/ml was added and incubated for 2 h at room temperature. The plates were then washed and incubated for 90 min at room temperature with 100 μl streptavidin-alkaline-phosphatase (Mabtech, Stockholm, Sweden) in a final dilution of 1/1000. Unbound conjugate was removed by another series of washes, and finally 100 μl of 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium substrate solution (Bio-Rad, Richmond, VA) were added and incubated in the dark until spots emerged (1 h). The color development was stopped by three washings with 200 μl tap water per well. After drying, the spots were counted in an ELISPOT reader (Biosys, Karben, Germany).

Statistical Analysis

Statistical analysis was performed by a paired Student's t test using SPSS for Windows 8.5 (SPSS, Chicago, IL). Values of $p < 0.05$ were considered statistically significant.

Results

Cross-linking of FcεRI alters the GM-CSF-IL-4-induced differentiation of human monocytes into DCs

At the beginning of culture, the cells used to generate DCs in a typical experiment consisted of 90% monocytes, as indicated by

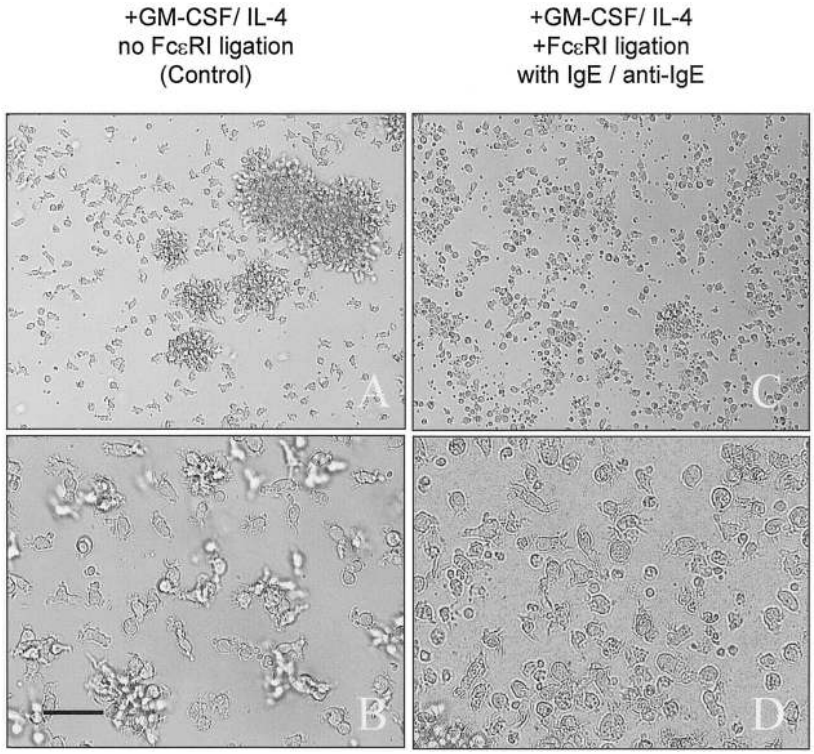

FIGURE 1. Morphological alterations induced by FcϵRI ligation. *A*, Cells from atopic donors cultured under standard conditions (i.e., GM-CSF and IL-4) are nonadherent, forming aggregates and displaying dendritic morphology. Magnification, ×20. *A*, Magnification, ×40. *B*, In contrast, FcϵRI-ligation at the beginning of the culture with IgE and anti-IgE leads to the generation of only a few cell aggregates of smaller diameter (*upper right panel*). Magnification, ×20. *C*, At higher magnification (×40), these cells appeared adherent, roundly shaped, and containing vacuoles (*D*) (bar size, 100 μm).

CD14 expression. The FcϵRI expression on monocytes was 9.1 ± 6.2% (n = 18) and 33.3 ± 16.3% (n = 28) positive cells for nonatopic and atopic individuals, respectively. The expression of CD23 on freshly isolated monocytes was <5% in all of the donors, as evaluated by flow cytometric analysis.

Upon control conditions with GM-CSF and IL-4, the cells rapidly became nonadherent and formed large clusters of DCs (Fig. 1, *A* and *B*) as reported by others (4, 5). In contrast, aggregation of FcϵRI on monocytes from atopic donors by IgE/anti-IgE or anti-FcϵRI mAbs at the time of the onset of culture with GM-CSF-IL-4 resulted in the appearance of a lower number of smaller aggregates of adherent, roundly shaped cells containing numerous vacuoles and resembling macrophages (Fig. 1, *C* and *D*).

Phenotypically, cells cultured under control conditions with or without addition of anti-CD23 or cells incubated with monoclonal IgG1 at the beginning of the culture all expressed high levels of CD1a and low levels of CD14 on day 6. This was in contrast to cells cross-linked with either human IgE/anti-IgE or anti-FcϵRI, which displayed lower levels of CD1a and high levels of CD14 (Fig. 2). Additionally, ligation of FcϵRI induced an increase of the mannose receptor expression but did not affect the expression of MHC class I (W6/32), MHC class II (HLA-DR), CD86 (B7-2), or CD80 (B7-1) at day 6 and of mature DCs (Fig. 3). Similarly, the expression of CD40, CD91, and CD68 was not significantly different at days 6 and 10 of culture (data not shown). By day 10, when they were fully mature, MoDCs lacked the mannose receptor but strongly expressed the DC-specific markers CD83 and p55 (25) (Fig. 3).

Some authors have reported that incubation only with monomeric IgE results in activation of monocytes and induces production of proinflammatory cytokines and up-regulation of adhesion molecule expression (26–28). To examine the effect of IgE binding to FcϵRI on DC differentiation, we cultured monocytes in the presence of 2 μg/ml hIgE and irrelevant second Abs in addition to GM-CSF and IL-4. This condition did not affect the generation of DCs with GM-CSF and IL-4, suggesting that cross-linking of FcϵRI was required for the suppressive effect. Thus, these findings demonstrate that ligation of FcϵRI on monocytes from atopic donors deeply affects the outcome of their differentiation.

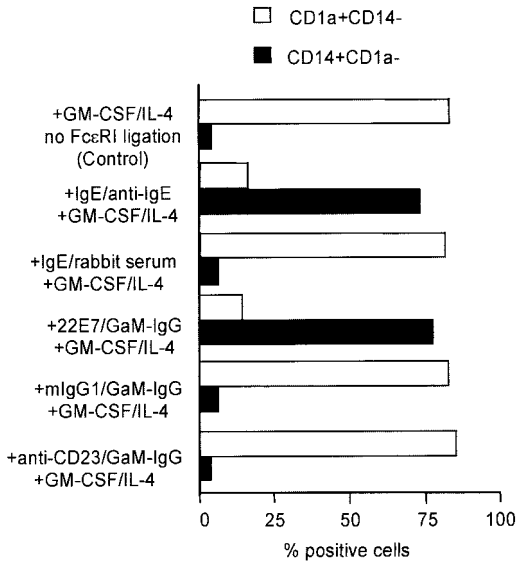

FIGURE 2. Alteration of MoDC generation is specific to FcϵRI. Cross-linking of the high affinity FcγR or the binding of hIgE to FcϵRI had no effect on GM-CSF-IL-4-induced differentiation. Monocytes were treated with 10 μg/ml 22E7 or MHM6 (anti-CD23) for 1 h or mIgG1 incubated with 5 μg/ml GaM-IgG in the presence of GM-CSF and IL-4 for 6 days. Alternatively, incubation of monocytes with 1 μg/ml hIgE for 1 h was followed by 20 μg/ml anti-human IgE or not, and then these cells were cultured with GM-CSF and IL-4. CD1a and CD14 expression was revealed by flow cytometric analysis, and representative results of four independent experiments are shown. Results are expressed as the percentage of $CD1a^+$ and $CD14^+$ cells. For all conditions, the SD ranged from 1 to 2%.

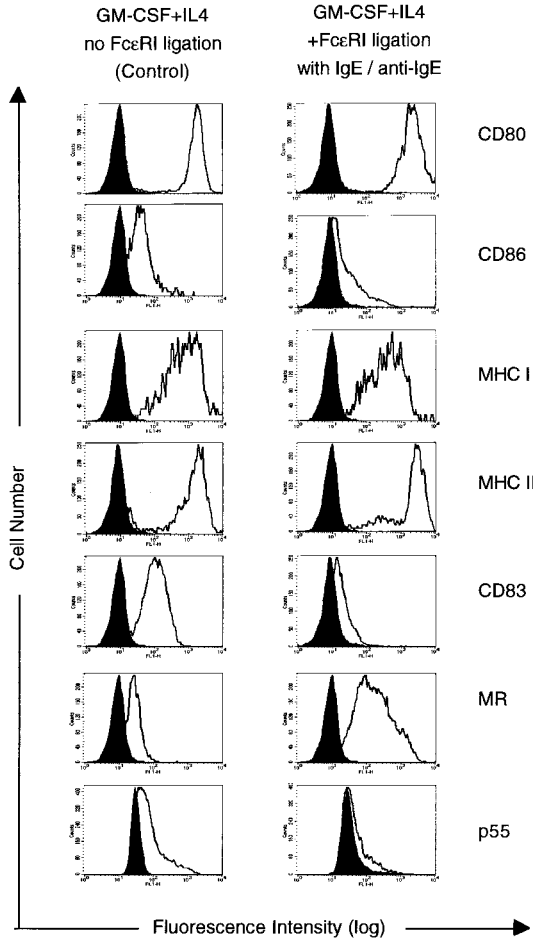

FIGURE 3. Cross-linking of FcεRI with IgE and anti-IgE prevents differentiation into DCs from human monocytes. Human monocytes from patients with AD were cultured either under standard conditions (GM-CSF-IL-4 for 6 days) or subjected to FcεRI cross-linking, then to standard culture, and finally analyzed by double-color fluorescence. The extracellular expression of the markers CD80, CD86, MHC class I (MHC I), MHC class II (MHC II), CD83, and the mannose receptor (MR), as well as the intracellular expression of permeabilized cells for p55 in correlation to the respective isotype control (filled black graph) is shown as one representative result of six independent experiments.

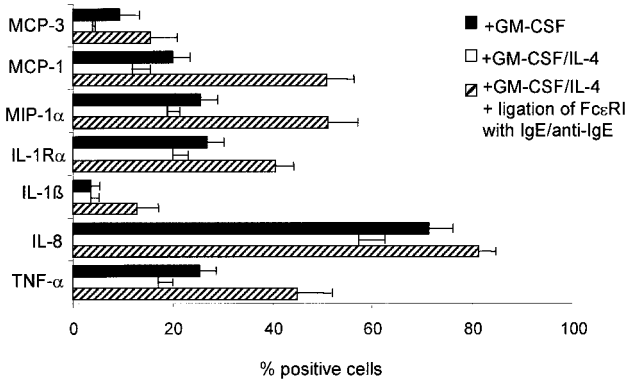

FIGURE 4. Capacity of producing proinflammatory cytokines and chemokines. FcεRI was cross-linked with IgE and anti-IgE at the initiation of the culture followed by 6 days in the presence of GM-CSF and IL-4 (▨). Alternatively, monocytes were cultured in the presence of GM-CSF and IL-4 (□) or GM-CSF (■) alone for 6 days. At that time, the cells were stimulated with 10 ng/ml PMA in the presence of brefeldin A for 24 h. Intracellular staining for cytokines and chemokines was conducted as described in *Materials and Methods*, and the percentages of positive cells of each cytokine were obtained by flow cytometric analysis. Representative figures of four independent experiments are shown.

Secretory and phagocytic properties of macrophages induced by FcεRI ligation

In the next step, aimed to unravel the functional characteristics of the cells generated upon FcεRI ligation, we examined their capacity to produce proinflammatory cytokines (IL-1β, IL-1Rα, and TNF-α) and chemokines (IL-8, MCP-1, MCP-3, and macrophage-inflammatory protein-1α) at a single-cell level by activation with PMA after 6 days culture. For this purpose, intracellular cytokine staining was used, and GM-CSF-induced macrophages were used as controls (26, 29). Interestingly, GM-CSF-IL-4-induced MoDCs produced markedly less proinflammatory cytokines and chemokines after stimulation with PMA compared with GM-CSF-induced macrophages (Fig. 4). However, the potency of producing proinflammatory cytokines and chemokines was significantly increased in the FcεRI-aggregated cells.

To verify that FcεRI ligation promotes the generation of macrophages, their phagocytic activity was evaluated by FITC-labeled, opsonized *E. coli*. Although MoDCs failed to display any phagocytic property by day 6, macrophage-like cells generated from monocytes by FcεRI ligation at the beginning of culture showed a high phagocytic capacity (Fig. 5) Interestingly, cross-linking of FcεRI at a later time point, i.e., at days 2 or 4, did not convert the cells to macrophages (data not shown). Viability staining with 7-amino actinomycin D and flow cytometric analysis revealed a viability of >95% of the cells in all of the different culture conditions.

Because a strong stimulatory activity is a hallmark of DCs, we tested the ability of the cells to generate in vitro upon GM-CSF-IL-4 and with or without FcεRI ligation in allogeneic MLR experiments. As expected, MoDCs obtained under standard conditions (GM-CSF-IL-4) showed strong allo-MLR-stimulating activity (Fig. 6). In contrast, cross-linking of FcεRI at the initiation of culture significantly down-regulated the stimulatory capacity in cells cultured with GM-CSF and IL-4. The stimulatory activity of

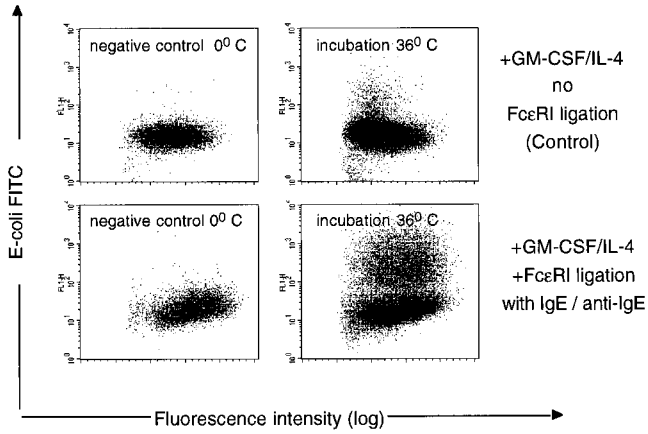

FIGURE 5. Phagocytic properties of macrophages induced by FcεRI ligation. Monocytes from donors with high FcεRI expression were subjected (*lower panels*) or not (*upper panels*) to receptor cross-linking with IgE and anti-IgE and cultured as described in *Materials and Methods*. The cells were then tested for their phagocytic activity using FITC-labeled *E. coli* and flow cytometry. This is one representative experiment; $n = 5$.

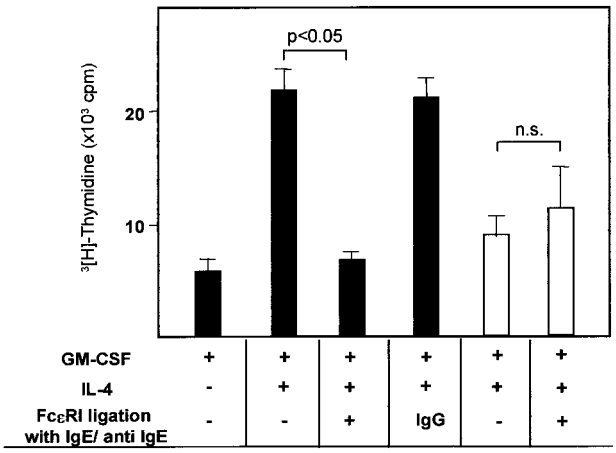

FIGURE 6. Cross-linking of FcεRI with IgE and anti-IgE suppresses the allostimulatory capacity of the cells cultured with GM-CSF and IL-4. FcεRI on human monocytes from atopic (■) and nonatopic donors (□) was cross-linked at the initiation of culture. These cells were cultured in the presence of GM-CSF and IL-4 for 6 days and used as stimulator cells. Cells were mixed at a ratio of 1:100 (APCs:responder cells) with purified responder allogeneic T lymphocytes (2×10^5 cells/well). Proliferation was assessed as [^{3}H]thymidine uptake during the last 18 h of a 4-day experiment. Representative results of four independent experiments are shown. Values are expressed as mean cpm ± SD of triplicate cultures (IgG, isotype control of the neutralizing anti-IL-10 mAb).

cells from nonatopic donors was not affected by addition of specific Ab. From these series of experiments, we can conclude that FcεRI-induced cells of atopic donors exhibit functional characteristics of typical macrophages.

FcεRI-induced secretion of IL-10 is a crucial factor for the generation of macrophages

In our experiments, the percentage of monocytes expressing FcεRI was always lower than the percentage of cells whose differentiation into DCs was prevented by the receptor ligation. This result suggests that, besides direct mechanisms, some soluble factors produced by monocytes after cross-linking of FcεRI might contribute to the prevention of the generation of MoDCs and lead to the generation of macrophages. To test this hypothesis, monocytes were cultured in the presence of GM-CSF and IL-4 and neutralizing mAbs against IL-1β, TNF-α, IL-6, M-CSF, or IL-10 (all at 10 μg/ml) or the respective IgG1 isotype control after aggregation of FcεRI. As a control, the neutralizing Abs were added to the standard conditions (GM-CSF and IL-4). There was no significant alteration of the phenotype or functional properties of the generated DCs induced by the Abs, and toxic effects were not detected by 7-amino actinomycin D-viability staining. The suppressive effect of FcεRI ligation on the generation of MoDCs with GM-CSF and IL-4 was considerably impaired by IL-10 neutralization, both phenotypically and functionally (Figs. 7 and 8). The treatment with mAbs against IL-1β, TNF-α, IL-6, or M-CSF significantly failed to influence the effect of FcεRI ligation on the generation of the cells. As a proof of concept, culture supernatants from monocytes with or without aggregation of FcεRI were tested for IL-10 release. Cells from nonatopic donors (low receptor expression) spontaneously produced low amounts of IL-10 that were not significantly increased upon receptor ligation (Fig. 9). In contrast, monocytes obtained from atopic donors (high receptor expression) spontaneously released significantly more IL-10, and this was dramatically increased upon receptor ligation ($p < 0.05$; $n = 3$).

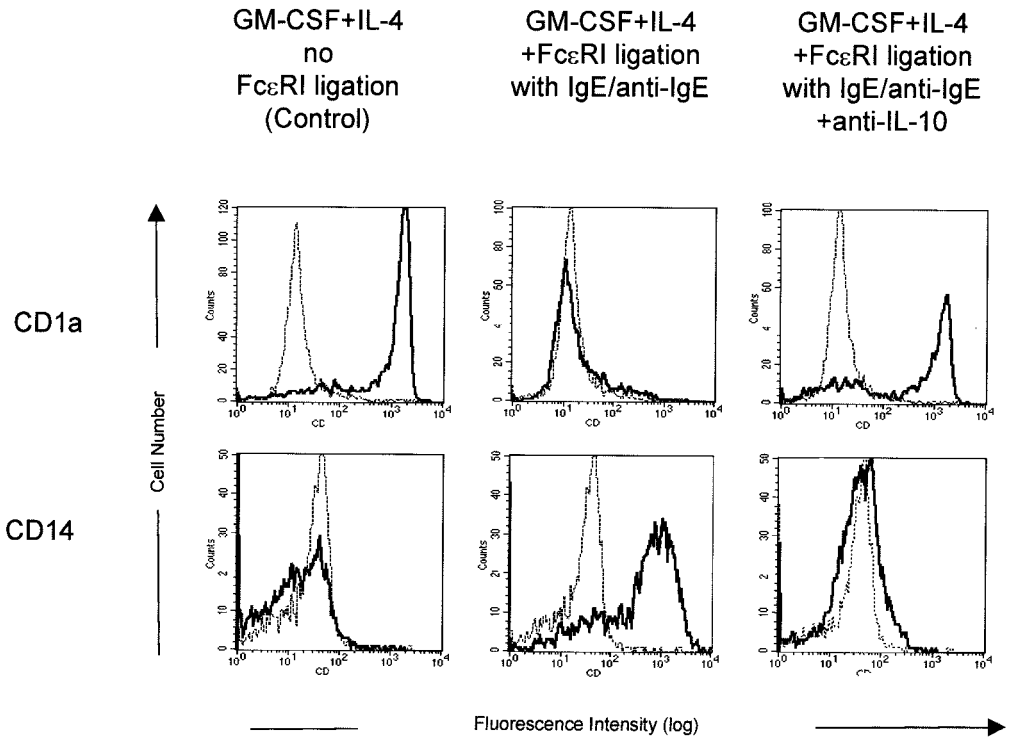

FIGURE 7. Inhibition of DC differentiation by the engagement of FcεRI with IgE and anti-IgE is suppressed by neutralization with anti-IL-10 mAb. After aggregation of FcεRI with IgE and anti-IgE, monocytes from atopic donors were cultured in the presence of 10 μg/ml anti-IL-10 mAb and GM-CSF and IL-4. After 6 days, CD1a$^+$ and CD14$^+$ expression of these cells (black line) was analyzed in correlation to the respective isotype control (dotted line). Representative results of six independent experiments are shown.

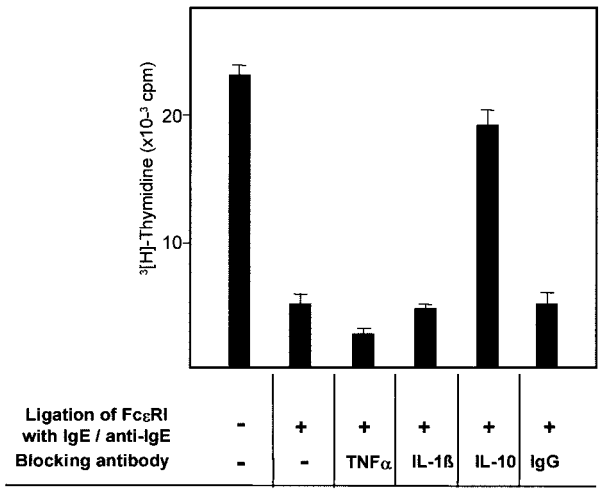

FIGURE 8. Suppressive effect of FcεRI ligation with IgE and anti-IgE in allogeneic MLRs is impaired considerably by IL-10 neutralization. Monocytes from atopic donors were cultured in the presence of 10 μg/ml anti-IL-10 in addition to GM-CSF and IL-4, with and without FcεRI ligation at the beginning of the culture for 6 days, and were used as stimulator cells (ratio of 1:100 APC:responder cells). MLR was performed as described in Fig. 4. Values are expressed as mean cpm ± SD of triplicate cultures. Representative results of four independent experiments are shown (IgG1, isotype control of the neutralizing anti-IL-10, anti-IL-1β, and anti-TNF-α mAbs).

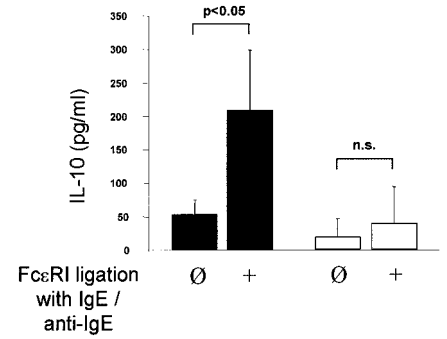

FIGURE 9. FcεRI ligation with IgE and anti-IgE induces the secretion of IL-10 by monocytes. Monocytes from atopic (■) and nonatopic donors (□) were subjected to FcεRI ligation and then cultured in the presence of GM-CSF and IL-4. After 24 h, the culture supernatants were analyzed by an IL-10-specific ELISA according to the manufacturer's protocol (R&D Systems). Values are expressed as the mean ± SD from three independent experiments (three atopic and three nonatopic donors).

In control experiments, when peripheral blood DCs or basophils were depleted from the monocytes as described above, no decrease in the IL-10 production of the cells could be detected. To ascertain that cross-linked monocytes of atopic donors are the major source of IL-10, cross-linking experiments with basophils, peripheral blood DCs, and purified monocytes of the same donor were done, and IL-10 production was measured in an ELISPOT assay. The number of IL-10-positive spots of purified monocytes was 600 ± 200, whereas the number of spots of cross-linked basophils of atopic donors was 50 ± 40, and no increase in the number of spots of peripheral blood DCs could be detected upon receptor ligation.

Discussion

Recently, evidence has been accumulated that the local cytokine environment and the presence of stimulatory signals determine whether monocytes will acquire DC or macrophage characteristics and function (30, 31). Monocytes cultured with either M-CSF or GM-CSF for several days differentiate into macrophages, whereas monocytes cultured with GM-CSF and IL-4 differentiate into immature DCs (4, 29, 32). Alternatively, CD40 ligation or calcium-ionophore treatment also leads to the generation of DCs from monocytes (33, 34), implying that distinct activation signals in monocytes induce DC generation. Unexpectedly, the present study demonstrates that activation signals induced by aggregation of FcεRI not only induce the production of cytokines such as IL-1β and TNF-α, but also IL-10, which prevents their differentiation in CD1a$^+$ MoDCs.

This observation is in line with some previous reports showing that the addition of IL-10 at the initiation of culture with GM-CSF and IL-4 or IL-13 results in the generation of macrophage-like cells that express high levels of CD14 and decreased levels of CD1a and are less efficient stimulators for T cells in MLRs than DCs (35). These effects were dose-dependent, and 200 pg/ml of IL-10 was sufficient for suppressing DC generation. Some speculate that IL-10 inhibits nuclear localization of the relB subunit of the NF-κB complex, whose expression has been shown to correlate with the differentiation of DCs but not with monocyte/macrophages (31, 36, 37).

Thus, FcεRI-induced IL-10 production by monocytes has profound consequences for their subsequent behavior and directs them toward macrophage-like cells. Other cytokines, such as IL-6 or M-CSF, have been shown to prevent the differentiation of CD34$^+$ progenitor cells to DCs (38, 39). However, our findings suggest that these cytokines are less critical in the control of the generation of DCs from monocytes by FcεRI ligation. IL-10 might suppress monocytes to produce some soluble factors required for maintaining DC characteristics, because this cytokine is known to inhibit the synthesis of a variety of proinflammatory cytokines by human monocytes (40). Alternatively, IL-10 itself may be able to exert a signal that drives monocytes to macrophages because human blood monocytes cultured with IL-10 for 18 h differentiate into CD16-positive macrophage-like cells (41). Recently, it was shown that IL-10 up-regulates mannose receptor expression and mannose receptor-mediated endocytosis of MoDCs and appears to stimulate pathways of Ag uptake in this way (42).

Cross-linking of FcεRI at the initiation of culture in the presence of brefeldin A only partly prevents DC generation with GM-CSF and IL-4, suggesting that FcεRI aggregation has some direct suppressive effect on DC differentiation (data not shown). Thus, in addition to extrinsic factors such as GM-CSF and IL-10, some intrinsic factor(s) whose expression is (are) up-regulated after cross-linking of FcεRI may be capable of regulating monocyte differentiation. One of the candidates is Bcl-2, an apoptosis-inhibiting protein. Experiments using transgenic mice that overexpress human Bcl-2 in monocytes demonstrate that Bcl-2-expressing monocytes spontaneously undergo macrophage differentiation without addition of M-CSF (43). Indeed, we observed that ligation of FcεRI on human monocytes rescues them from apoptosis by up-regulating the expression of Bcl-2 and Bcl-X_L (20). Whether a survival signal is sufficient for macrophage maturation or whether Bcl-2 can play some other role in cell differentiation remain to be clarified.

In the present observation, CD1a$^+$ MoDCs did not shift to macrophages by cross-linking of FcεRI on days 2 or 4. Several nonmutually exclusive events may explain this phenomenon. It is possible that the level of expression of FcεRI is rapidly down-regulated during culture to obtain DCs in the absence of its ligand

(28) and, consequently, the FcεRI-mediated signal may not be sufficient for further preventing MoDC generation. Another possibility would be that, at this stage of differentiation, MoDCs become unresponsive to IL-10. This would be in line with observations of Allavena et al., who reported that IL-10 failed to induce the generation of cells of a macrophage phenotype when it was added to cells already deeply engaged in DC differentiation, e.g. by day 6 (35). Furthermore, kinetic experiments have shown that the suppressive effect of IL-10 on MLRs with MoDCs becomes progressively weaker, implying that the sensitivity of DCs to IL-10 is down-modulated during differentiation (44). The observation that culture of GM-CSF-IL-4-induced DCs in cytokine-free-medium leads to cells rapidly becoming macrophages rules out the possibility that already-differentiated DCs cannot convert to macrophages. Thus, all these findings strongly suggest that IL-10 acts on monocytes essentially before their commitment to become DCs.

In the present report, we provide the first evidence that, besides its known functions in the context of Ag-presentation, FcεRI plays a pivotal role in the modulation of the differentiation of DCs from atopic donors. This may be of relevance for in vivo conditions, particularly in the context of atopic disorders. It is established that aeroallergens can penetrate into the skin of atopic individuals due to increased permeability or to erosions and/or excoriations. In contrast, food allergens are taken up by the gastrointestinal tract and are found in the peripheral blood. In addition, there are considerable amounts of specific IgE against autoantigens in sera of AD patients (45, 46). As shown herein, engagement of FcεRI with these allergens could lead to drive monocytes into macrophages, which then may play some yet-to-be-defined regulatory role in the atopic inflammation. Under the present experimental conditions, the capacity to produce proinflammatory cytokines was greater in FcεRI-engaged macrophage-like cells than that in in vitro-generated DCs. Thus, infiltrating macrophages could amplify the inflammatory reaction following repeated Ag exposure by secretion of proinflammatory cytokines and chemokines. This would in turn contribute to the establishment and/or maintenance of chronic lesions in allergic diseases or to enhancement of host defense function against infection.

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