

Peripheral Blood Dendritic Cells Express FcεRI as a Complex Composed of FcεRIα- and FcεRIγ-Chains and Can Use This Receptor For IgE-Mediated Allergen Presentation¹

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Originally limited to basophils and mast cells, the spectrum of high affinity IgE receptor (FcεRI)-bearing cells has expanded recently to include Langerhans cells, dermal dendritic cells (DC), monocytes, and eosinophils. As a result of studies on the distribution, structure, and function of FcεRI on APCs, we discovered a minor nonbasophil, nonmonocyte PBMC population that can bind IgE via FcεRI. This receptor occurs on the surface of these cells as a multimeric structure containing FcεRIα- and FcεRIγ-chains but, unlike its counterpart on basophils, lacking FcεRIβ. Further experiments revealed that these FcεRIαγ-expressing cells closely resemble peripheral blood DC by immunophenotype (HLA-DR^{high}, HLA-DQ^{high}; CD4⁺, CD11a⁺, CD32⁺, CD33⁺, B7/2 (CD86)⁺; CD11b^{low}, CD14^{low}, CD40^{low}, CD54^{low}, CD64^{low}) and cell morphology. These features allowed us to isolate FcεRI-expressing DC from the peripheral blood and to investigate their immunostimulatory properties. We found FcεRI-positive DC to be efficient stimulators of both primary (allogeneic MLR) and FcεRI/IgE-dependent, secondary T cell responses at low cell numbers. Thus, FcεRI-expressing DC may not only amplify established type I allergic immune reactions but, unlike FcεRI-positive semiprofessional APCs, may be able to prime naive T cells to common and/or cryptic epitopes of IgE-reactive Ags. *The Journal of Immunology*, 1996, 157: 607–616.

The high affinity receptor for IgE (FcεRI) is expressed on basophils/mast cells, epidermal Langerhans cells (1, 2)/dermal dendritic cells (DC)³ (1, 3), monocytes (4), and eosinophils (5). Molecular studies demonstrated that, on both rodent and human basophils/mast cells, FcεRI occurs as a multimeric immune recognition receptor composed of one IgE-binding α-chain noncovalently attached to one β- and one pair of γ-chains (6). As opposed to its well-known function on basophils/mast cells (i.e., the induction of cellular degranulation and release of various mediators), FcεRI on APC functions as an allergen-focusing structure that, in the case of monocyte-enriched PBMC of atopic individuals, can efficiently amplify allergen presentation in an IgE-dependent manner (7). To better characterize the spectrum of

FcεRI-expressing APC within the peripheral blood, we screened PBMC for HLA-DR⁺ (i.e., presumably Ag-presenting) cells that can bind monomeric IgE via FcεRI but fail to express monocyte-specific markers (e.g., CD14). We found that, in addition to basophils and monocytes, a third type of FcεRI-expressing cells circulates in the peripheral blood of both atopic and healthy individuals. We have (immuno-) phenotypically characterized this cell population, resolved the subunit composition of FcεRI as expressed on the surface of these cells, and finally, comparatively analyzed the IgE-dependent and IgE-independent immunostimulatory functions of monocytes and of this novel cellular subset.

Materials and Methods

Monoclonal antibodies and immunoreactants

The following FITC-conjugated mouse mAbs were used in this study: anti-CD1a (OKT6; Ortho Pharmaceuticals, Raritan, NJ), anti-CD1c (7C6; provided by Drs. O. Majdic and W. Knapp, Institute of Immunology, Vienna, Austria), anti-CD4 (Leu3a; Becton Dickinson, Mountain View, CA), anti-CD11a (6B7; Institute of Immunology), anti-CD11b (LM2; American Type Culture Collection (ATCC), Rockville, MD), anti-CD11c (3.9; Sera-Lab, Sussex, U.K.), anti-CD14 (LeuM3; Becton Dickinson), anti-CD16 (Leu11a; Becton Dickinson), anti-CD19 (Leu12; Becton Dickinson), anti-CD23 (IOB8; Immunotech, Marseille, France), anti-CD25 (anti-IL-2R; Becton Dickinson), anti-CD32 (IV.3 Fab; Medarex, West Lebanon, NH), anti-CD33 (4D3; Institute of Immunology), anti-CD34 (HPCA-2; Becton Dickinson), anti-CD40 (5C3; PharMingen, San Diego, CA), anti-CD45 (HLe-1; Becton Dickinson), anti-CD45RA (Leu18; Becton Dickinson), anti-CD45RO (UCHL-1; An der Grub, Bio Research GmbH, Kaumberg, Austria), anti-CD54 (IOL54; Immunotech), anti-CD64 (32.2 F(ab')₂; Medarex), anti-CD86 (FUN-1; PharMingen), anti-CD117 (17F11; An der Grub), anti-HLA-DR (SK10; Becton Dickinson), and anti-HLA-DQ (Leu10; Becton Dickinson). Phycoerythrin (PE)-conjugated mouse mAbs included anti-CD80 (BB1/B7; Becton Dickinson) and anti-CD86 (IT2.2; PharMingen). Purified mAbs used were chimeric (human Fc/mouse Fab) IgE anti-4-hydroxy-3-nitrophenacetyl (cIgE anti-NP; Serotec, Oxford, U.K.), HECA-452 (rat anti-cutaneous lymphocyte-associated Ag (CLA); kindly provided by Dr. Louis Picker, Dallas, TX), mAbs 15-1 and 19-1

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³ Abbreviations used in this paper: DC, dendritic cells; aa, amino acids; ACDU, automatic cell deposit unit; AD, atopic dermatitis; CHO, Chinese hamster ovary; cIgE, chimeric IgE anti-4-hydroxy-3-nitrophenacetyl; CO, control; MACS, magnetic cell separation; MFC, mean fluorescence channel; NP, nitrophenacetyl; PB-DC, peripheral blood dendritic cells; PE, phycoerythrin; PerCP, peridinin chlorophyll; RT, reverse transcriptase; SA, streptavidin; TCC, T cell clone.

(both mouse anti-FcεRIα; Ref. 1), MHM6 (mouse anti-CD23; Dakopatts), and DAKO-HLA-DR/α (mouse anti-HLA-DR; Dakopatts). Isotype control mAbs included MOPC-21 (mouse IgG1; Sigma Chemical Co., St. Louis, MO), VIAP (mouse IgG1 anti-alkaline phosphatase; Institute of Immunology), FITC- or PE-labeled mouse IgG1, IgG2a, and IgG2b (Becton Dickinson), and rat IgM (Southern Biotechnology, Birmingham, AL). Anti-HLA-DR PerCP was obtained from Becton Dickinson. Anti-FcεRIβ Abs were raised against amino acids 1 to 16 of human FcεRIβ by immunizing rabbits with chicken serum albumin-coupled peptides. After serum collection, FcεRIβ-specific rabbit Ig was affinity purified on peptide-coupled Sepharose 6B (Pharmacia, Uppsala, Sweden). Streptavidin-PE (SA-PE) and SA-PE/Cy5 conjugates were from Becton Dickinson and Dakopatts, respectively. FITC-conjugated F(ab)₂ fragments of affinity-purified mouse anti-rat Ig Abs were provided by Dr. Adelheid Elbe, Division of Immunology, Allergy, and Infectious Diseases (Vienna, Austria).

Cell preparations and cell lines

E⁻-mononuclear cells (E⁻-PBMC) from healthy nonatopic or atopic donors, i.e., birch pollen-allergic patients (as defined by clinical criteria, skin prick test, and radioallergosorbent test) and patients suffering from atopic dermatitis (AD), were prepared by Ficoll-Hypaque (Pharmacia) density-gradient centrifugation of heparinized venous blood, followed by depletion of SRBC-binding T lymphocytes. Thereafter, residual T cells, B cells, and NK cells were removed by anti-CD3 (anti-Leu4; Becton Dickinson), anti-CD19 (anti-Leu12; Becton Dickinson), and anti-CD56 (anti-Leu19; Becton Dickinson) immunomagnetic depletion using sheep anti-mouse Ig-coated paramagnetic beads (Dynabeads M-450; Dynal, Oslo, Norway). In certain experiments, these lymphocyte-depleted PBMC were further separated on the basis of anti-CD11b immunoreactivity into cells highly enriched for monocytes (CD11b⁺ lymphocyte-depleted PBMC) and into a population largely depleted of anti-CD11b-reactive cells. Briefly, lymphocyte-depleted PBMC were reacted with anti-CD11b-coated paramagnetic particles (MACS; Milteny Biotec, Bergisch-Gladbach, Germany) in PBS plus 1% BSA (Behringwerke AG, Marburg, Germany). Thereafter, cell suspensions (1×10^7 cells/ml) were run over separation columns attached to a magnet (Mini-MACS; Milteny Biotec). Non-bead-bound cells were recovered by two sequential washing steps. Bead-bound cells were eluted by removing the magnet and injecting the columns twice with 2 ml of PBS. Naive CD4⁺ T cells were prepared from E⁺-PBMC by anti-CD8 (anti-Leu2; Becton Dickinson) and anti-CD45RO (UCHL-1) immunomagnetic depletion using sheep anti-mouse Ig-coated Dynabeads M-450 (Dynal). T cell purity as determined by flow cytometric analysis was consistently >95%.

Chinese hamster ovary (CHO) cells were obtained from ATCC. CHO cells expressing surface FcεRIαγ complexes (CHOαγ) have been described elsewhere (1). Stable transfectants expressing FcεRIαβγ (CHOαβγ) were obtained by cotransfecting CHO cells with human FcεRIα, FcεRIβ, and FcεRIγ cDNAs using methods similar to those previously described (1).

Three-color immunofluorescence analysis

To release in vivo bound IgE molecules, lymphocyte-depleted PBMC were subjected to lactic acid treatment, as previously described (8). After preincubation in 500 μg/ml of affinity-purified human serum IgG (Chemicon, Temecula, CA) for 30 min at room temperature, cells (5×10^5 in 50 μl) were exposed simultaneously to biotinylated mAb 15-1 or cIgE (each 5 μg/ml), to anti-HLA-DR PerCP (5 μg/ml), and to anti-LeuM3 (5 μg/ml) FITC or 1 to 5 μg/ml of one of the other FITC-conjugated mAbs. Cell-bound biotinylated mAb 15-1 or cIgE was visualized by SA-PE (1 μg/ml). In selected experiments, cells were incubated for 30 min with 50 μg/ml of mAb 15-1, MHM6, or MOPC-21 before the exposure to biotinylated cIgE. When PE-labeled mAbs were used in conjunction with anti-HLA-DR FITC and biotinylated 15-1, the cell-bound biotin groups were detected with SA-PE/Cy5 (1 μg/ml). Cellular fluorescence and light-scatter parameters were analyzed on a FACScan (Becton Dickinson). The specific reactivity of FITC- or PE-labeled mAbs with a given cell type was expressed as the Δ-mean fluorescence channel number (Δ-MFC: MFC of the reactivity of the FITC/PE-labeled mAb - MFC of the reactivity of an isotype- and fluorochrome-matched control mAb). FcεRI-dependent cIgE binding to a given cell type was assessed by subtracting the MFC of biotinylated cIgE reactivity in the presence of 50 μg/ml of mAb 15-1 from the MFC of cIgE reactivity of positive cells in the presence of 50 μg/ml of an IgG1 control mAb (MOPC-21). Statistical significances were determined using the paired Student's *t* test, and a *p* value of <0.05 was considered to be statistically significant.

Immunohistochemical studies on lymphocyte-depleted PBMC subpopulations

Lymphocyte-depleted PBMC, stained with anti-LeuM3 FITC and biotinylated cIgE plus SA-PE, were sorted into IgE^{high} (basophils) and IgE^{low} CD14⁻ subpopulations using a FACStar^{Plus} (Becton Dickinson) equipped with an automatic cell deposit unit (ACDU; Becton Dickinson). A quantity amounting to 2×10^4 cells of each population was sorted directly into RPMI 1640 (Life Technologies, Paisley, Scotland) plus 10% heat-inactivated human AB serum (kindly provided by the Institute of Blood Group Serology, Vienna, Austria)-containing wells of chamber slides (Nunc, Roskilde, Denmark). Cells were harvested either immediately or following culture for 36 h at 37°C. Recovered cells were spun gently onto adhesion slides (Bio-Rad, Richmond, CA), fixed in methanol (Merck, Darmstadt, Germany) for 15 min at -20°C, quenched with 1% normal horse serum (Vector Laboratories, Burlingame, CA), and exposed to 2 μg/ml of either the IgG1 control mAb MOPC-21 or an anti-HLA-DR mAb (DAKO-HLA-DR/α; Dakopatts). After three washings in PBS, cell-bound mAbs were conjugated with biotinylated horse anti-mouse IgG (Vector Laboratories) and visualized using avidin/biotinylated peroxidase complexes (ABC Elite Vectastain Kit; Vector Laboratories) and 3-amino-9-ethylcarbazole (Sigma Chemical Co.). The samples were counterstained with Harris' hematoxylin (Merck) and finally embedded in Glycergel (Dakopatts).

Immunoprecipitation and immunoblot experiments

Cells were exposed to cIgE (5 μg/ml, 30 min), extensively washed, and reacted with diisopropyl-fluorophosphate (Sigma Chemical Co.; 5 mM, 5 min). After centrifugation, cell pellets were solubilized in 1% Brij 96 (Sigma Chemical Co.)-containing Tris lysis buffer (20 mM, pH 8.2) supplemented with 140 mM NaCl, 2 mM EDTA, 1 mM iodoacetamide, 1 mM PMSF, aprotinin, leupeptin (both 10 μg/ml), 0.1 mM quercetin, 0.1 mM *N*-tosyl-L-phenylalanine chloromethyl ketone, 0.1 mM *N*-*p*-tosyl-L-lysine chloromethyl ketone, and 0.1 mM *N*-CBZ-L-phenylalanine chloromethyl ketone (all from Sigma Chemical Co.). Lysates were kept on ice for 30 min, extensively precleared, and then reacted with anti-IgE- or BSA-coupled Sepharose for at least 4 h at 4°C. Covalent coupling of proteins to CNBr-activated Sepharose beads (Pharmacia) was performed according to the manufacturer's guidelines. Lysate-exposed Sepharose beads were washed twice in ice-cold PBS; immobilized material was eluted with SDS sample buffer and analyzed by immunoblotting, as previously described (4). Briefly, aliquots of eluates were subjected to 12% SDS-PAGE and blotted onto nitrocellulose. Membranes were blocked with PBS/0.05% Tween-20 containing 10% BSA or 5% milk proteins for at least 6 h. Thereafter, membranes were exposed to biotinylated mAb 19-1, biotinylated IgG2a, polyclonal rabbit anti-FcεRIβ, or polyclonal rabbit anti-FcεRIγ. Ab binding to immobilized proteins was detected with peroxidase-conjugated streptavidin (1:2,000; Amersham International, Buckinghamshire, U.K.) or goat anti-rabbit Ig (1:50,000; Bio-Rad) as the second step reagent.

RT-PCR on sorted cell populations

A quantity amounting to 1×10^4 HLA-DR⁺CD14⁺, HLA-DR⁺CD14⁻FcεRI⁺, HLA-DR⁻CD14⁻FcεRI⁺, and HLA-DR⁻CD14⁻FcεRI⁻ lymphocyte-depleted PBMC was sorted into single PCR tubes, each containing 150 μl of lysis buffer (Dynabeads mRNA Direct Kit; Dynal), using the ACDU facility of the FACStar^{Plus}. For control purposes, lysates of CHOαγ and CHOαβγ cells were prepared. All lysates were frozen immediately to -20°C for 18 h. After thawing, the samples were vortexed for 1 min and reacted with 20 μl of Dynabeads oligo(dT)₂₅ for 5 min at room temperature to allow hybridization of mRNA poly(A) tails to the beads. After several washings, immobilized poly(A) mRNA was detached from the beads by adding 20 μl of elution buffer (Dynabeads mRNA Direct Kit) to the reaction mixture. An aliquot of 5 μl of eluted mRNA (containing mRNA of 2.5×10^3 cells) was reacted in the absence or presence of 10 U of MuLV RT (Boehringer Mannheim Corp., Mannheim, Germany) with FcεRIα (TAC AGT AAT GTT GAG G)-, FcεRIβ (TTT GAG TTC TTC CCC AGC TC)-, or FcεRIγ (GGT GGG ACA AGA GAG TGA GAC T)-specific oligonucleotide RT primers (1 pmol/μl), 40 U RNasin (Promega Corp., Madison, WI), and 1 mM dNTPs (Boehringer Mannheim Corp.). After incubation at 37°C for 1 h, cDNA synthesis was stopped by heating the samples to 70°C for 15 min. Thereafter, cDNAs were precipitated, washed in 70% ETOH, and resuspended in 20 μl of H₂O. Ten microliters of individual samples were diluted to a final volume of 50 μl in PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTPs) containing 0.05 U/μl of Taq polymerase (AmpliTaq; Perkin-Elmer Corp., Norwalk, CT) and 1.25 pmol/μl of the individual oligonucleotide primers. Primer pairs used include: FcεRIα (3'-TAC AGT AAT GTT GAG GGG CTC AG; 5'-CTG TTC TTC GCT CCA GAT GGC GT),

FcεRIβ (3'-GGT GAG AAA CAG CAT CAT C; 5'-AAA TCT TGC TCT CCC ACA GG), and FcεRIγ (3'-GCA TGC AGG CAT ATG TGA TGC C; 5'-CCA GCA GTG GTC TTG CTC TTA C). The cycling was started with denaturation at 94°C for 40 s, annealing at 55°C for 30 s, and elongation at 70°C for 40 s. For the first 25 cycles, the annealing temperature was decreased by 0.2°C per cycle and then kept constant at 50°C for 10 additional cycles. PCR was performed on a GenAmp thermocycler (PCR System 9600; Perkin-Elmer Corp.). A 10-μl aliquot of each PCR product was subjected to electrophoresis on ethidium bromide (Boehringer Mannheim Corp.)-stained 2% agarose (Life Technologies) gels. To ensure the specificity of amplification, several PCR products were sequenced directly on an automated sequencer (373A DNA sequencer; Applied Biosystems, Foster City, CA). Briefly, DNA in a 37-μl aliquot of the original amplification products was precipitated and subjected to dyedexy sequencing using the DyeDeoxy Terminator Sequencing Kit (Applied Biosystems) according to the manufacturer's recommendations. Sequencing primers were identical with those used for PCR, and their concentrations were optimized empirically for the sequencing reaction. After phenol/chloroform extraction and EtOH precipitation, sequencing products were electrophoresed on 6% polyacrylamide/8.3 M urea sequencing gels. Sequencing of both DNA strands of amplification products revealed perfect alignment of the PCR-derived FcεRIα, FcεRIβ, and FcεRIγ DNA sequences with published data.

Proliferation assays

Primary MLR, lactic acid-treated, and γ-irradiated (1500 rad) lymphocyte-depleted PBMC were flow sorted according to their anti-CD14, anti-HLA-DR, and anti-FcεRI immunoreactivity. Graded numbers (0.3 to 10×10^3) of individual cell populations were deposited directly into individual wells of 96-well round-bottom microtiter plates (Costar, Cambridge, MA). As responders, 5×10^4 allogeneic naive CD4⁺ T cells were added to each well. All MLR cultures were performed in RPMI 1640 (Life Technologies) supplemented with 10% heat-inactivated human AB serum in a total volume of 200 μl/well. Cultures were incubated for 5 days before pulsing with 0.5 μCi of [³H]thymidine (Amersham International) per well for additional 16 h. Incorporation of [³H]thymidine was measured by liquid-scintillation spectroscopy. The results are expressed as the mean ± SD of cpm obtained in duplicate cultures. Background counts of controls (responder T cells or sorted stimulator cells alone) were always <500 cpm.

Allergen-specific T cell proliferation

ACDU-sorted cells, anti-CD11b-depleted, or anti-CD11b-enriched lymphocyte-depleted PBMC were used as stimulator cells and dispensed into individual wells of 96-well round-bottom microtiter plates (Costar). Cells were incubated with 10 μg/ml of IgG1 or mAb 15-1 before exposure to 1 μg/ml of cIgE or medium and the indicated concentrations of recombinant major birch pollen allergen rBet v 1 (Mayerhofer, Linz, Austria) chemically conjugated to NP, as previously described (7). Thereafter, 5×10^4 cells of autologous Bet v I-specific T cell clones (TCC) were added as responders. TCC used in this study included WD24 (epitope specificity Bet v 1; amino acids (aa), 143–156), TF1 M (Bet v I; aa, 94–108), and TF7B (Bet v I; aa, 13–24; Ref. 7). All proliferation assays were performed in serum-free UltraCulture medium (BioWhittaker, Walkersville, MD). Cultures were incubated for 48 h and processed as described for the MLR experiments. Background counts of controls (APC, APC plus Ag, TCC, APC plus TCC, TCC plus Ag) were always <500 cpm.

Results

Lymphocyte-depleted PBMC contain a population of HLA-DR-positive, CD14-negative cells that bind IgE via FcεRI

To determine whether IgE receptor-bearing cell types other than lymphocytes, basophils, or monocytes exist in the peripheral blood, lymphocyte (B cell (anti-CD19), T cell (anti-CD3), and NK cell (anti-CD56))-depleted PBMC from healthy individuals were stained with biotinylated monomeric IgE followed by SA-PE, anti-CD14 FITC, and anti-HLA-DR PerCP. Flow cytometric analysis revealed the existence of two IgE receptor-expressing nonmonocyte (i.e., CD14-negative) cell populations that can be discriminated on the basis of their IgE-binding and anti-HLA-DR-staining properties (Fig. 1*a*). One cellular subset has the IgE^{low}CD14[−] phenotype (red; Fig. 1, *a* and *b*) and stains brightly with an anti-HLA-DR mAb; the other population consists of IgE^{high}CD14[−] cells (green; Fig. 1, *a* and *b*) that fail to express HLA-DR and, thus, qualify as basophils (Fig. 1*b*). To investigate the nature of the IgE

receptor(s) used by the HLA-DR⁺CD14[−] and the HLA-DR[−]CD14[−] populations, lymphocyte-depleted PBMC from normal individuals were treated with mAbs to either FcεRIα (mAb 15-1) or FcεRII (mAb MHM6) before exposure to biotinylated IgE. As seen in Figure 1*c*, the IgE-binding capacity of HLA-DR⁺CD14[−] cells (red histogram) is abolished by pretreatment with anti-FcεRIα mAb (green histogram). In contrast, preincubation of the cells with anti-CD23 mAb had no effect on the IgE-binding capacity of either cell type (data not shown). Identical mAb inhibition results were observed with HLA-DR[−]CD14[−] cells greatly enriched for basophils (Fig. 1*d*).

In a next series of experiments, we asked whether IgE-binding HLA-DR⁺CD14[−] cells also exist in the peripheral blood of atopic individuals and, if so, whether these cells differ in their FcεRI-dependent IgE-binding capacity from those of healthy controls. When analyzing cells from 10 AD patients and 10 healthy controls (CO), we found 1) that all of the investigated samples contained IgE-binding HLA-DR⁺CD14[−] cells (data not shown); 2) that, at least under the experimental conditions used, FcεRI was the only receptor for monomeric IgE used by these cells (Fig. 1 and data not shown); and 3) that the FcεRI-dependent IgE-binding capacity of these cells was significantly higher in the AD than in the CO group (Fig. 2). As expected from our previous observations (10), CD14⁺HLA-DR⁺ monocytes of AD patients were superior to those of CO individuals with regard to their FcεRI-dependent IgE-binding capacity. Nevertheless, monocytes of AD patients were approximately 10-fold less effective in IgE binding than the HLA-DR⁺CD14[−] cells (Fig. 2). An increased IgE-binding capacity of leukocytes from atopic vs nonatopic individuals was also seen with basophils, but this difference was not statistically significant. Taken together, our results demonstrate that, in addition to basophils, a nonmonocyte HLA-DR⁺ cell population exists in the peripheral blood of healthy and atopic individuals that can bind monomeric IgE via FcεRIα.

HLA-DR-positive, CD14-negative PBMC lack FcεRIβ and express FcεRI as a multimeric complex composed of FcεRIα and FcεRIγ chains

To gain detailed information on the molecular composition of cell surface-expressed FcεRI on HLA-DR⁺CD14[−] PBMC as compared with basophils, we performed immunoprecipitation and immunoblotting experiments on cell populations purified from the peripheral blood of healthy individuals. Knowing that, unlike basophils, IgE-binding HLA-DR⁺CD14[−] PBMC do not express the myeloid Ag CD11b (see below), we removed CD11b-reactive basophils and monocytes from lymphocyte-depleted PBMC and, by doing so, generated a cell population greatly enriched for HLA-DR⁺CD14[−] IgE-binding cells. For comparison, we depleted the basophil-enriched cell populations from monocytes by plastic adherence. Basophils as well as HLA-DR⁺CD14[−] PBMC were exposed to monomeric IgE, washed, and solubilized in 1% Brij 96 lysis buffer that preserves the holotetrameric αβγ₂ structure of FcεRI on basophils and CHOαβγ cells (Fig. 3*a* and data not shown). Cell surface-derived IgE-bound proteins were purified by anti-IgE immunoprecipitation, blotted onto nitrocellulose, and identified by Abs against FcεRIα, FcεRIβ, or FcεRIγ. Results obtained demonstrate that IgE receptor complexes from HLA-DR^{high}CD14[−] PBMC contain FcεRIα and FcεRIγ chains (Fig. 3*a*). Most strikingly, FcεRI complexes of HLA-DR^{high}CD14[−] PBMC were devoid of anti-FcεRIβ immunoreactivity, whereas FcεRIβ protein could be detected easily within anti-IgE precipitates from basophil and CHOαβγ surfaces (Fig. 3*a*). To determine

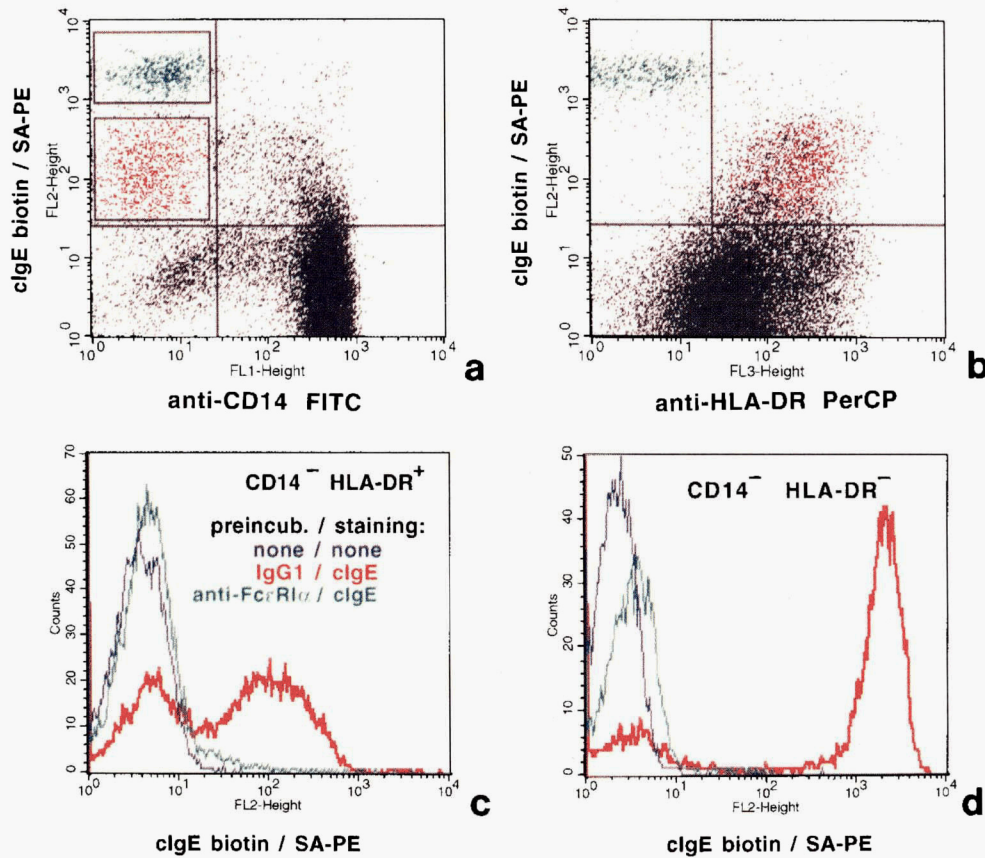


FIGURE 1. Lymphocyte-depleted PBMC contain a population of HLA-DR-positive, CD14-negative cells that bind IgE via FcεRI. Three-color flow cytometry analysis of anti-CD14 FITC (*a*; x-axis), clgE/SA-PE (*a* and *b*; y-axis), and anti-HLA-DR PerCP (*b*; x-axis) immunoreactivity of lymphocyte-depleted PBMC from a healthy individual. Two distinct populations of IgE-binding cells can be discriminated on the basis of their clgE-binding capacities. IgE^{low} and IgE^{high} cells are depicted in red and green, respectively. *c* and *d*, Analysis of the nature of the clgE-binding structure(s) of CD14[−]HLA-DR⁺ and CD14[−]HLA-DR[−] lymphocyte-depleted PBMC. The clgE reactivity of CD14[−]HLA-DR⁺ (*c*) and CD14[−]HLA-DR[−] cells (*d*) pretreated with either the anti-FcεRIα mAb 15-1 (green histogram) or an isotype-matched control mAb (red histogram) is depicted. The blue histograms represent the background fluorescence levels in the absence of clgE. Representative experiment.

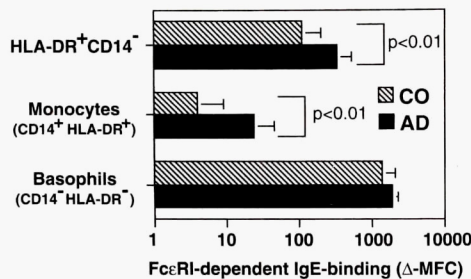


FIGURE 2. Quantitative analysis of the FcεRI-dependent IgE-binding capacity of HLA-DR⁺CD14[−], HLA-DR⁺CD14⁺ (i.e., monocytes), and HLA-DR[−]CD14[−] (i.e., cells greatly enriched for basophils) lymphocyte-depleted PBMC from healthy and atopic individuals. FcεRI-dependent IgE-binding capacities of the individual cell populations were calculated as described in *Materials and Methods*. Data represent the mean $\pm$ SD of Δ -MFC values obtained with cells from 10 healthy control individuals (CO) and 10 AD patients.

whether FcεRIβ protein, although absent from cell surface-expressed FcεRI, occurs within the cytoplasm of HLA-DR^{high}CD14[−] PBMC, we subjected these cells to Nonidet P-40 lysis that yields optimal cellular solubilization (data not shown). In these extracts, FcεRIβ protein was detected neither by anti-FcεRIβ Western blotting nor by anti-FcεRIβ immunoprecipitation (data not shown). To investigate whether the absence and presence of

FcεRIβ protein in HLA-DR^{high}CD14[−] PBMC and basophils, respectively, are due to different FcεRIβ transcriptional activity in these two cell types and/or are related to the atopic status of a given individual, we performed PCR experiments on poly(A⁺) RNA-derived cDNA from 2.5×10^3 flow-sorted FcεRI⁺CD14[−]HLA-DR⁺ cells and FcεRI⁺CD14[−]HLA-DR[−] basophils from two healthy donors and two AD patients. For control purposes, we prepared RNA-derived cDNA from lymphocyte-depleted and flow-sorted CD14⁺HLA-DR⁺ monocytes, FcεRI[−]CD14[−]HLA-DR[−] cells, and FcεRIαγ- or FcεRIαβγ-transfected CHO cells. Using cells from an atopic donor, we found that FcεRI⁺CD14[−]HLA-DR⁺ cells (as well as FcεRIαγ-transfected CHO cells) coexpress mRNA encoding FcεRIα and FcεRIγ, but lack FcεRIβ-specific transcripts (Fig. 3*b*). In contrast, basophils from the same donor (as well as FcεRIαβγ-transfected CHO cells) synthesize not only FcεRIα and FcεRIγ mRNA, but also FcεRIβ-specific transcripts. The high purity of the sorted cell populations is demonstrated by the lack of detectable FcεRIα and FcεRIβ transcripts in FcεRI[−]CD14[−]HLA-DR[−] cells that contain CD34⁺ progenitor cells and other rare lineage Ag-negative cell types (data not shown). As seen with FcεRI⁺CD14[−]HLA-DR⁺ cells, sorted FcεRI⁺CD14⁺HLA-DR⁺ monocytes were found to contain mRNA encoding FcεRIα and FcεRIγ, but to lack FcεRIβ-specific transcripts. In keeping with this observation, we failed to detect, by immunoblotting, FcεRIβ in lysates of FcεRIα⁺/

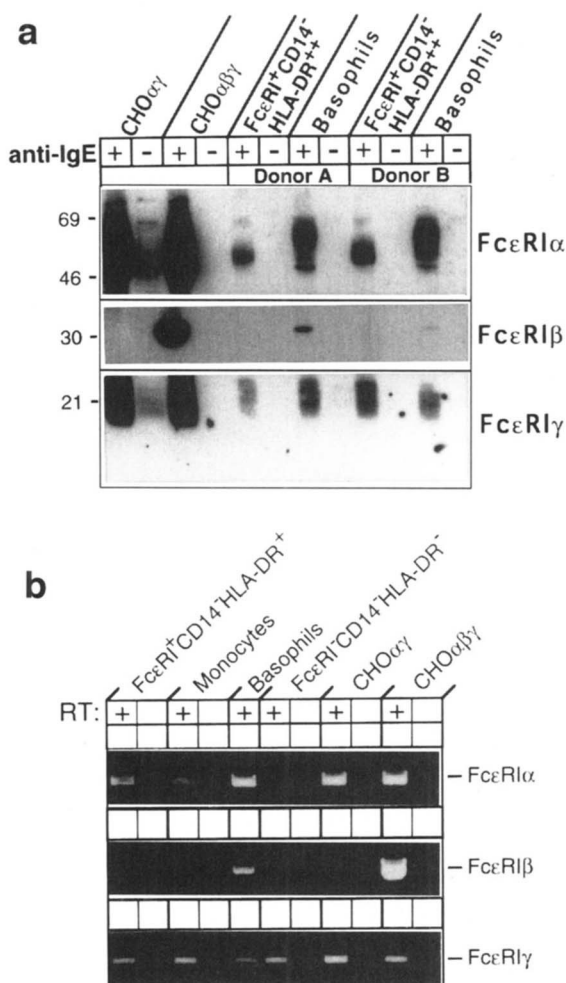


FIGURE 3. *a*, HLA-DR⁺CD14⁻ lymphocyte-depleted PBMC express cell surface Fc ϵ RI complexes composed of Fc ϵ RI α and Fc ϵ RI γ , but devoid of Fc ϵ RI β . Fc ϵ RI⁺HLA-DR⁺CD14⁻ PBMC- and basophil-enriched cell suspensions (containing 3×10^6 cells each) were prepared from lymphocyte-depleted PBMC of two healthy donors. These cell populations as well as CHO cells transfected with Fc ϵ RI α and Fc ϵ RI γ (CHO $\alpha\gamma$) or with Fc ϵ RI α , Fc ϵ RI β , and Fc ϵ RI γ (CHO $\alpha\beta\gamma$) were exposed to cIgE and solubilized in 1% Brij 96-containing lysis buffer. Lysates containing the cIgE-bound, cell surface-derived Fc ϵ RI complexes were reacted with anti-IgE (+) or BSA-coupled Sepharose (-); immobilized material was eluted from the beads, subjected to 12% SDS-PAGE, and blotted onto nitrocellulose. Membranes were exposed to biotinylated anti-Fc ϵ RI α mAb 19-1 (*top panel*), polyclonal rabbit anti-Fc ϵ RI β (*middle panel*), and polyclonal rabbit anti-Fc ϵ RI γ (*bottom panel*). First step Ab reactivity with membrane-bound proteins was detected with peroxidase-conjugated streptavidin or goat anti-rabbit Ig as second step reagent. *b*, Fc ϵ RI⁺HLA-DR⁺CD14⁻ cells contain mRNA encoding Fc ϵ RI α and Fc ϵ RI γ , but lack Fc ϵ RI β -specific transcripts. Poly(A⁺) RNA-derived cDNA of 2.5×10^4 flow-sorted Fc ϵ RI⁺HLA-DR⁺CD14⁻, HLA-DR⁺CD14⁺ (monocytes), Fc ϵ RI⁺HLA-DR⁺CD14⁻ (basophils), or Fc ϵ RI⁺HLA-DR⁺CD14⁻ cells was subjected to Fc ϵ RI α -, Fc ϵ RI β -, or Fc ϵ RI γ -directed PCR in the presence or absence of MuLV RT. For control purposes, cDNA from CHO $\alpha\gamma$ and CHO $\alpha\beta\gamma$ cells was processed and analyzed in parallel.

Fc ϵ RI γ ⁺ monocytes (data not shown). When we performed RT-PCR experiments on cells from another atopic donor and two healthy control individuals, we obtained identical results, with the exception that more PCR cycles were needed to amplify Fc ϵ RI α from the weakly IgE-reactive monocytes of the two normal donors. Taken together, these data demonstrate that Fc ϵ RI⁺CD14⁻HLA-

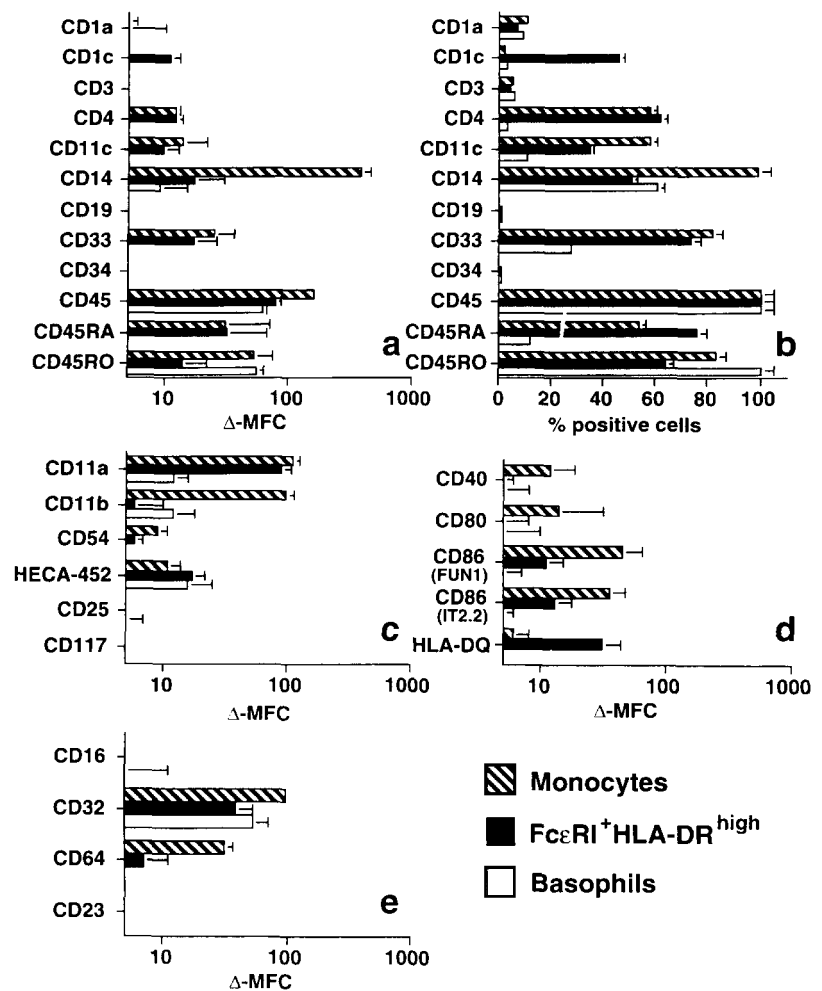
DR⁺ cells, unlike basophils, do not synthesize Fc ϵ RI β , but assemble Fc ϵ RI on their cell surface as a receptor complex containing Fc ϵ RI α and Fc ϵ RI γ subunits.

Fc ϵ RI⁺HLA-DR⁺CD14⁻ cells exhibit the phenotypic and functional features of peripheral blood dendritic cells (PB-DC)

To determine the immunophenotype of the newly defined Fc ϵ RI⁺CD14⁻HLA-DR⁺ cells as compared with monocytes and basophils, we performed three-color immunofluorescence flow cytometry experiments using mAbs against Fc ϵ RI α and HLA-DR, and a panel of mAbs recognizing lineage-specific Ags, adhesion/costimulatory molecules, cytokine receptors, or FcR. The gating criteria for the three cell populations investigated were Fc ϵ RI^{low}HLA-DR^{low} (monocytes), Fc ϵ RI⁺HLA-DR⁻ (basophils), and Fc ϵ RI⁺HLA-DR^{high}. As expected, cells gated for the monocyte criteria expressed approximately 50 times more CD14 than the Fc ϵ RI⁺HLA-DR^{high} cells or the Fc ϵ RI⁺HLA-DR⁻ basophils (Fig. 4*a*). The analysis of lineage Ag expression revealed that the Fc ϵ RI⁺HLA-DR^{high} cells uniformly express CD45, but are devoid of anti-CD1a, anti-CD3, anti-CD19, and anti-CD34 immunoreactivity (Fig. 4*a*). Despite certain similarities to monocytes (e.g., expression of CD11c and CD33), Fc ϵ RI⁺HLA-DR^{high} cells display only low levels of the myeloid Ags CD14 (Fig. 4*a*) and CR3/CD11b (Fig. 4*c*), and express CD1c (Fig. 4*a*) and HLA-DQ (Fig. 4*d*). In contrast to basophils, they abundantly express CD4, CD11c, CD33, and CD45RA (Fig. 4*a*), but are virtually devoid of CD11b (Fig. 4*c*). When we analyzed the proportions of Fc ϵ RI⁺HLA-DR^{high} cells that stain with mAbs against the various lineage/differentiation Ags, we found that subpopulations of Fc ϵ RI⁺HLA-DR^{high} cells can be defined on the basis of CD1c, CD11c, CD45RO, and CD45RA expression (Fig. 4*b* and data not shown). Furthermore, Fc ϵ RI⁺HLA-DR^{high} cells display pronounced anti-CD11a immunoreactivity, but low levels of ICAM-1 (CD54; Fig. 4*c*). Interestingly, Fc ϵ RI⁺HLA-DR⁺ cells, monocytes, and basophils react with the mAb HECA-452 that recognizes the skin-specific homing receptor, cutaneous lymphocyte-associated Ag (Fig. 4*c*). CD25 or SCF receptor (CD117) expression was not evident on any of the three cell types (Fig. 4*c*). While Fc ϵ RI⁺HLA-DR^{high} cells displayed only low levels of the costimulatory molecules B7/1 (CD80), B7/2 (CD86), and CD40 when analyzed immediately after isolation from peripheral blood (Fig. 4*c*), marked up-regulation of B7 receptor expression occurred upon in vitro culture (data not shown). In contrast to monocytes, Fc ϵ RI⁺HLA-DR^{high} cells bear virtually no high affinity IgG receptors (Fc γ RI). The only other type of Fc γ R found on these cells was a low affinity receptor for IgG, Fc γ RII (CD32), whereas CD16 and CD23/Fc ϵ RII expression levels were below the detection limit (Fig. 4*e*). The latter finding is in keeping with the above observation that anti-CD23 mAbs are not efficient in inhibiting IgE binding to HLA-DR⁺CD14⁻ lymphocyte-depleted PBMC. Apart from the previously mentioned up-regulated Fc ϵ RI-dependent IgE binding of HLA-DR⁺CD14⁻ lymphocyte-depleted PBMC of atopics (Fig. 2), we found no significant immunophenotypic differences between Fc ϵ RI⁺HLA-DR^{high} cells from AD patients and those of healthy control subjects (data not shown). Taken together, these data strongly suggest that the Fc ϵ RI⁺HLA-DR^{high} cell population circulating in the peripheral blood of healthy and atopic individuals displays an immunophenotype closely resembling that described for freshly isolated, noncultured PB-DC (9–13).

To see whether this Fc ϵ RI⁺HLA-DR^{high} cell population also displays the cardinal morphologic features of PB-DC, we flow

FIGURE 4. The immunophenotype of FcεRI⁺HLA-DR⁺ cells closely resembles that of PB-DC. Lineage Ag (a and b), adhesion/costimulatory molecule/cytokine receptor/HLA-DQ (c and d), and FcR expression (e) by FcεRI^{high}HLA-DR^{high} cells (full columns), FcεRI^{low}HLA-DR^{low} monocytes (shaded columns), and FcεRI^{high}HLA-DR⁻ basophils (open columns). Mean values ± SD of Δ-MFC intensity (a, and c through e; x-axis) and percentage of positive cells (b; x-axis) for a given Ag (a through e; y-axis) and cell population were obtained using PBMC from eight healthy donors.



sorted lymphocyte-depleted PBMC with regard to their IgE-binding capacity and their anti-CD14 reactivity into IgE^{low}CD14⁻ cells (see red cell population, Fig. 1a) and IgE^{high}CD14⁻ basophils (see green cell population, Fig. 1a). Sorted cells were immobilized onto adhesion slides and were then immunostained for HLA-DR expression either immediately after sorting or following a 36-h incubation in granulocyte-macrophage CSF-supplemented medium (Fig. 5). As seen in Figure 5A, IgE^{low}CD14⁻ cells homogeneously react with anti-HLA-DR mAb, whereas no cell-bound immunoreactivity was noted when an isotype-matched control mAb was used (Fig. 5b) or when we probed the IgE^{high}CD14⁻ basophil population for HLA-DR expression (data not shown). Before culture, the cells sorted for the criteria IgE^{low}CD14⁻ appeared as round cells containing moderate amounts of cytoplasm and complex, often polylobulated nuclei (Fig. 5a). Following culture in vitro, these cells experience profound (immuno-) morphologic changes, i.e., 1) an overall increase in cell size (Fig. 5c), 2) pronounced HLA-DR expression in juxtanuclear positions and, to lesser extent, on the cell surface (Fig. 5c), and 3) the appearance of delicate dendritic membrane protrusions as visualized by phase-contrast microscopy (Fig. 5d).

To see whether the FcεRI⁺HLA-DR⁺CD14⁻ cells also fulfill functional criteria of DC, we tested these cells for their capacity to induce proliferation of allogeneic naive (i.e., CD45RO⁻) CD4⁺ T cells. Lymphocyte-depleted PBMC were stained with mAb 15-1, anti-HLA-DR, and anti-CD14, and graded numbers of HLA-DR⁺CD14⁻ cells (supposedly containing all subpopulations of

PB-DC), FcεRI⁺HLA-DR⁺CD14⁻ cells, and HLA-DR⁺CD14⁻ monocytes were sorted directly into individual wells of tissue culture plates to which allogeneic responder cells were added. Results shown in Figure 6 demonstrate that FcεRI⁺HLA-DR⁺CD14⁻ cells have the capacity to vigorously stimulate naive CD4⁺ T cells (representative results, four independent experiments). The magnitude of the observed response at least equals that induced by the unfractionated HLA-DR⁺CD14⁻ DC population, but greatly exceeds that elicited by HLA-DR⁺CD14⁺ monocytes (Fig. 6). Taken together, these results demonstrate that, by phenotype and function, FcεRI⁺HLA-DR⁺CD14⁻ PBMC are IgE-binding DC that circulate in the peripheral blood of both healthy and atopic individuals.

PB-DC can present allergen in an FcεRI/IgE-dependent manner

Using a recently described allergen presentation model that allows the targeting of haptenized (i.e., NP-conjugated) recombinant birch pollen allergen (rBet v 1-NP) to Fc-IgE receptors via NP-specific monomeric IgE (cIgE) (7), we investigated 1) whether purified DC populations from atopics can mediate FcεRI/IgE-dependent allergen presentation to allergen-specific TCC and, if so, 2) whether the IgE-dependent presentation capacity of these cells differs quantitatively from that of the same donor's monocytes. Lymphocyte-depleted PBMC from atopic donors were MACS sorted with regard to their anti-CD11b immunoreactivity into 1) cell populations highly enriched for IgE-binding DC, but largely depleted of

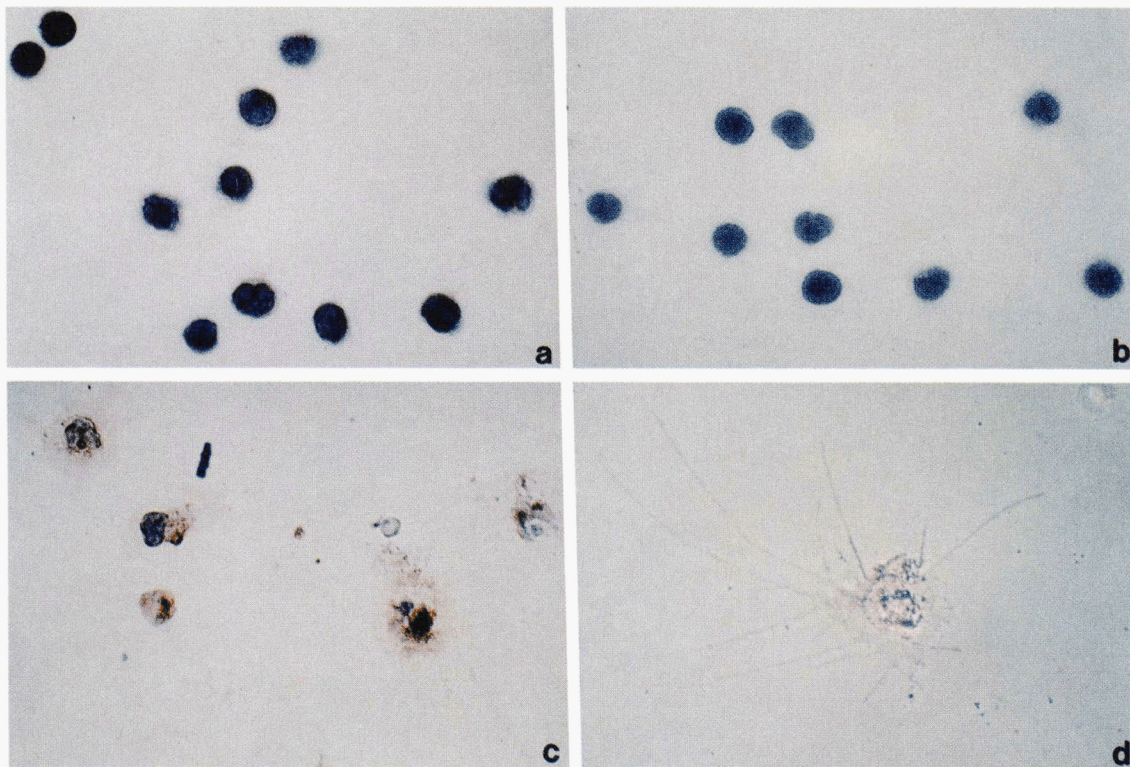


FIGURE 5. Upon culture *in vitro*, IgE-binding, CD14⁺, anti-HLA-DR-reactive cells acquire a dendritic morphology. Cells were sorted into individual wells of chamber slides according to their cIgE reactivity and CD14 expression using gating criteria, as shown in Figure 1a for the cell population in red (i.e., IgE^{low} and CD14⁺). Sorted cells were harvested either immediately after their isolation (a and b), or after a 36-h incubation period in granulocyte-macrophage CSF-supplemented, 10% human serum-containing medium (c and d), were mounted onto adhesion slides, fixed, and stained with an anti-HLA-DR (a and c) or an isotype-matched control mAb (b). d, Phase microscopy of sorted cells upon culture *in vitro*.

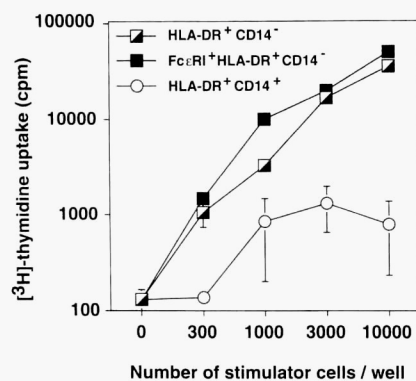


FIGURE 6. FcεRI⁺HLA-DR⁺CD14⁺ cells are potent stimulators of naive allogeneic CD4⁺ T cells. A quantity amounting to 0.3 to 10 × 10³ irradiated and sorted HLA-DR⁺CD14⁺ cells (semi-open squares), FcεRI⁺HLA-DR⁺CD14⁺ cells (closed squares), and HLA-DR⁺CD14⁺ monocytes (open circles) was cocultured with 5 × 10⁴ allogeneic naive CD4⁺ T cells for 5 days before pulsing with [³H]thymidine for additional 16 h. The results are expressed as the mean ± SD cpm of duplicate cultures (ordinate). Representative results as obtained in one of four experiments.

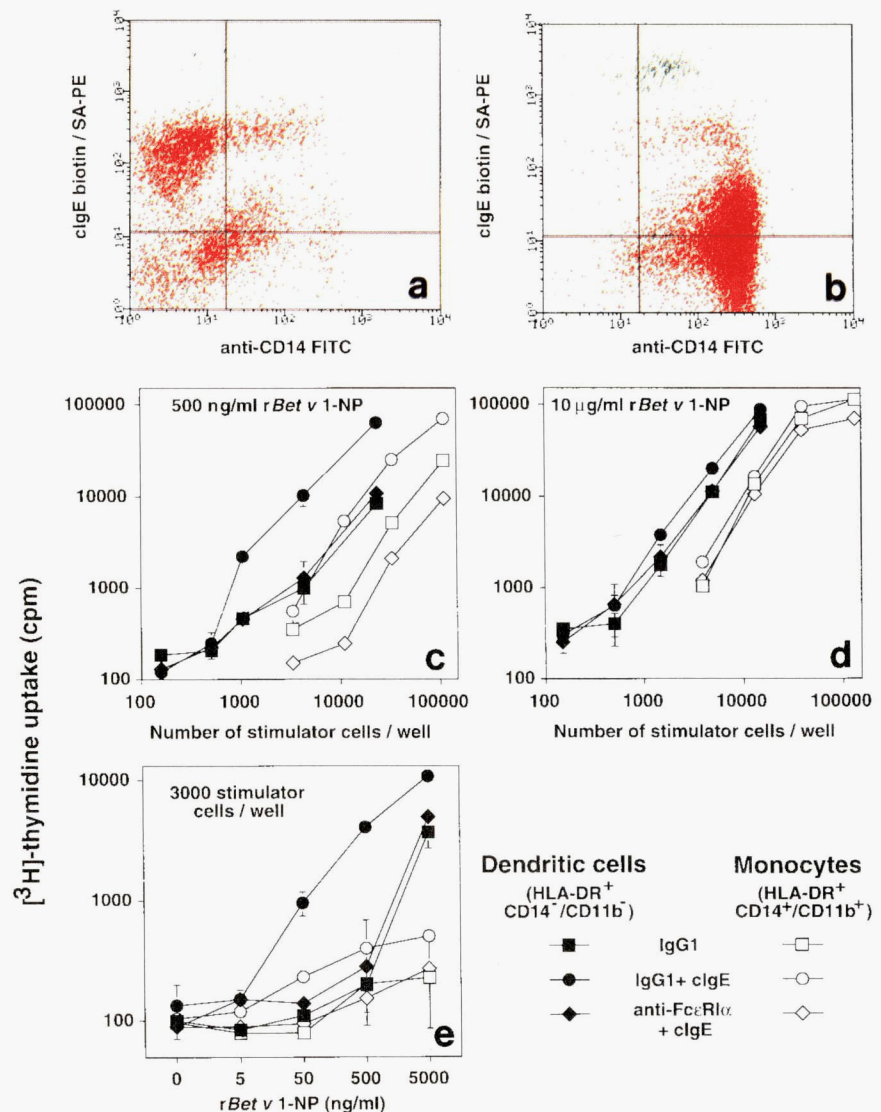
CD14⁺HLA-DR⁺ monocytes (CD11b⁺ cells; Fig. 7a), and 2) cell populations devoid of DC, but consisting mostly of monocytes (CD11b⁺ cells; Fig. 7b). The addition of hapten-specific IgE to DC or monocyte cultures results in a 5- to 10-fold amplified proliferative TCC response at low concentrations of the haptenized allergen rBet v 1 (500 ng/ml, Fig. 7c; one of two independent experiments with similar results). On a cell-to-cell basis, however,

DC and monocytes differ from each other in their relative efficacy to elicit IgE-dependent allergen-specific T cell responses. Whereas as few as 1 to 5 × 10³ DC were sufficient to mount significant IgE-dependent proliferative T cell responses, as many as 1 to 5 × 10⁴ monocytes were needed to obtain a similar effect (Fig. 7c). The addition of the anti-FcεRIα mAb 15-1 reduces both DC- and monocyte-induced TCC proliferation to levels seen with allergen in the absence of hapten-specific IgE (Fig. 7c). These findings demonstrate that both cell types use FcεRI as their principal IgE receptor mediating IgE-dependent allergen presentation. When 3 × 10³ highly purified, flow-sorted CD14⁺HLA-DR⁺ lymphocyte-depleted PBMC were used as stimulators for Ag-titration experiments, we found that the IgE/FcεRI-dependent pathway of allergen presentation by DC is indeed a dose-dependent and saturable mechanism that levels off at allergen concentrations that allow sufficient allergen uptake in an IgE-independent manner (Fig. 7e). Taken together, our results demonstrate that FcεRI⁺ DC can present allergen in an FcεRI/IgE-dependent manner and are approximately 10 times more potent than monocytes, on a per cell basis, in stimulating allergen-specific, secondary T cell responses.

Discussion

Only recently we have gained an understanding of certain of the Ag-uptake mechanisms operative in various types of APC. These include nonselective (i.e., fluid-phase micro- or macropinocytosis-mediated (14)) and substance-specific (e.g., mIg- (15–17), FcR- (7, 18–23), and C-type lectin receptor-mediated (24, 25)) interiorization functions. In particular, specific Ag recognition and internalization capabilities of professional APC, i.e., DC, are of major

FIGURE 7. PB-DC use FcεRI to present allergen to HLA-DR-restricted T cells in an IgE-dependent manner. Lymphocyte-depleted PBMC from birch pollen-allergic donors were MACS sorted into CD11b[−] (a) and CD11b⁺ (b) cell populations, and were stained with anti-CD14 FITC (x-axis), biotinylated clgE/SA-PE (y-axis), and anti-HLA-DR PerCP (HLA-DR⁺ and HLA-DR[−] cells are depicted in red and green, respectively) to control purification quality. c and d, Indicated numbers of DC (closed symbols) or monocytes (open symbols) thus purified were reacted with IgG1 (squares), IgG1 plus NP-specific clgE (circles), or mAb 15-1 (anti-FcεRIα) plus NP-specific clgE (diamonds), followed by the addition of 500 ng/ml (c) or 10 μg/ml NP-conjugated rBet v 1 (d). In e, 3 × 10³ HLA-DR⁺CD14[−] cells (closed symbols) and 3 × 10³ HLA-DR⁺CD14⁺ monocytes (open symbols) were directly ACUDU sorted into individual wells and exposed to the indicated concentrations of NP-conjugated rBet v 1 (x-axis) in the presence of IgG1 (squares), IgG1 plus NP-specific clgE (circles), or mAb 15-1 (anti-FcεRIα) plus NP-specific clgE (diamonds). T cells of TCC TF1 M (c) or WD24 (d and e) were added as responders, and [³H]thymidine uptake was measured (cpm; ordinate).



interest since they may have profound implications on the elicitation, and perhaps even the diversification of Ag-specific T cell responses. In this work, we show that a structural variant of the high affinity receptor for IgE (FcεRI) is expressed on a PB-DC population, and that these cells use this receptor as an efficient allergen-focusing structure for enhanced allergen presentation.

Our contention that the newly defined population of FcεRI⁺HLA-DR⁺CD14[−] cells is DC is based on 1) their typical DC morphology, 2) an immunophenotype that has been assigned previously to freshly isolated PB-DC (HLA-DR^{high}, HLA-DQ^{high}, CD4⁺, CD11a⁺, CD32⁺; CD11b^{low}, CD14^{low}, CD40^{low}, CD54^{low}, CD64^{low}, B7/2 (CD86)^{low}, B7/1 (CD80)[−] (9–13)), and 3) their capability to induce proliferation of naive CD4⁺ T cells. The distinctive nature of these cells is highlighted by the fact that FcεRI⁺ DC can be discriminated qualitatively from basophils by their high HLA-DR or HLA-DQ and from monocytes by their low CD14, CD11b, or CD64 expression (Fig. 4). Whereas homogeneous expression patterns were noted with most differentiation Ags tested, pronounced CD1c, CD11c, CD33, CD45RO, or CD45RA expression was confined to subpopulations of FcεRI⁺ DC. This indicates that FcεRI⁺ DC are a functionally heterogeneous cell population that includes the CD11c[−], CD33^{low}, CD45RA⁺, and

the CD11c⁺, CD33^{high}, CD45RO⁺ PB-DC subsets (12, 13). While data from O'Doherty et al. (13) imply that the latter cell population represents an advanced maturational step of the former subset, recent observations by Zhou and Tedder indicate that the CD1c⁺, CD11c⁺, CD33^{high}, CD45RO⁺ PB-DC can also originate from mature peripheral blood monocytes (26). Our further observation that a non-IgE-binding, FcεRI[−]HLA-DR⁺CD14[−] PBMC population also exists (Fig. 1c) that is highly stimulatory in the primary allogeneic MLR (M. Schmitt-Egenolf, manuscript in preparation) and, thus, fulfills the criteria of DC, strengthens the concept that a remarkable phenotypic, and possibly functional, DC heterogeneity exists in the peripheral blood.

The coimmunoprecipitation and molecular experiments on FcεRI of PB-DC and basophils show directly that DC assemble FcεRI as a multimeric receptor containing one IgE-binding FcεRIα chain and FcεRIγ chains, presumably as a homodimer, but lacking the FcεRIβ subunit, whereas basophils (and mast cells) express FcεRI in the tetrameric αβγ₂ configuration (Fig. 3). In view of these data and our previous findings with Langerhans cells (1) and monocytes (4), it is conceivable that APC generally use the FcεRIαγ₂ receptor type as their IgE-binding moiety, whereas the IgE receptor-bearing effector

cells of immediate type allergic immune responses express the conventional tetrameric holoreceptor. The possibility that FcεRIα₂ complexes are expressed and are functional even in the absence of FcεRIβ gains support from transfection experiments. These studies demonstrated that transfection of human FcεRIα and FcεRIγ constructs leads to cell surface expression of FcεRI in the absence of FcεRIβ (27, 28), and that this receptor is capable of mediating tyrosine phosphorylation of at least certain cellular substrates (29), calcium mobilization (4, 28), and internalization of receptor-bound ligands (30). In analogy to FcεRIαγ transfectants, FcεRI-expressing APC undergo protein tyrosine phosphorylation (31) and calcium mobilization upon receptor cross-linking (4, 30). However, the possibility exists that FcεRI of native APC contains FcεRIβ-related structures, e.g., products of the CD20/FcεRIβ/HTM₄ gene family (32), that substitute for FcεRIβ and, perhaps, modify the functional repertoire of FcεRI on these cells. Such a molecule may be critically involved in signal transduction and, possibly, in the intracellular targeting of allergens taken up via FcεRI. The experimental conditions established in this study, i.e., the precipitation of FcεRI complexes from the surface of small numbers of professional APC, should now allow the purification and identification of such hitherto unknown adapter molecules.

Using an allergen presentation model that uses recombinant haptenized (NP-conjugated) major birch pollen allergen (rBet v 1-NP) and NP-specific monomeric IgE, we found that DC as well as monocytes elicit FcεRI/IgE-amplified proliferative TCC responses (Fig. 7, *c* and *d*), demonstrating that both cell types express functionally competent FcεRI. The further observation that FcεRI⁺ DC far exceed monocytes in their capacity to present allergen to primed T cells in both IgE-dependent and IgE-independent manners demonstrates their indigenously superior stimulatory activity not only for primary, but also for secondary immune reactions. The possibility that FcεRI/IgE-dependent allergen presentation by DC is of relevance for the occurrence/perpetuation of atopic inflammatory tissue reactions (e.g., allergic late-phase reactions and AD) is supported by the observations of accumulating FcεRI⁺ DC-like cells in late phase reactions (S. Wichlas, unpublished) and AD (R. Klubal, manuscript submitted) lesions. It is conceivable that these cells are derived from IgE-bound PB-DC, although their immigration into the tissue has yet to be proven. In this scenario, the abundance of allergen-specific, and possibly tissue-penetrating, serum IgE along with the up-regulated FcεRI-dependent IgE-binding capacity of PB-DC of atopic individuals may synergistically favor efficient IgE loading of these cells and, subsequently, IgE-dependent allergen presentation to skin-infiltrating T cells. It is presently unclear whether FcεRI-expressing DC can exert IgE-dependent functions in healthy individuals with IgE serum concentrations at approximately 200 ng/ml. Clarification of this issue will require investigations on the Ag specificities of naturally occurring IgE molecules, the function of FcεRI on healthy individuals' DC, and the analysis of their immunostimulatory properties as compared with those of atopics.

DC, as opposed to monocytes, are APC with the ability to stimulate primary T cell responses (9). It might be the FcεRI⁺ DC described in this work that has the property to activate and prime naive T cells to hitherto unrecognized epitopes of IgE-reactive Ags/allergens. It can be further envisaged that IgE-dependent allergen uptake may influence the repertoire of allergen-derived peptides generated and presented by DC. As previously described for Ig-dependent Ag presentation by B cells (33), such a mechanism should relate closely to the binding specificity(-ies) of the recognizing IgE molecule(s). Thus, the presentation of epitopes located outside the IgE recognition site may be greatly favored, whereas

the HLA class II peptide assembly of those located within the binding site of IgE may be suppressed. In summary, FcεRI/IgE-dependent allergen uptake by DC may both quantitatively and qualitatively modulate allergen presentation and have profound implications on the magnitude and diversification of allergen-specific T cell responses.

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