

Expression of Functional High Affinity Immunoglobulin E Receptors (FcεRI) on Monocytes of Atopic Individuals

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Summary

Suggestive evidence indicates that immunoglobulin E (IgE)-dependent activation of mononuclear phagocytes plays an important pathogenic role in allergic tissue inflammation. Prevailing opinion holds that low affinity IgE receptors are the relevant IgE-binding structures on monocytes/macrophages and that functional events occurring after cross-linking of membrane-bound IgE on these cells are mediated by these receptors. Here we demonstrate that peripheral blood monocytes can bind monomeric IgE via the high affinity IgE receptor (FcεRI) and that FcεRI expression on these cells is upregulated in atopic persons. Further, we demonstrate that, upon monocyte adherence to substrate, bridging of monocyte FcεRI is followed by cell activation. We propose that direct interaction of multivalent allergen with FcεRI⁺-bound IgE on mononuclear phagocytes results in cell signaling via FcεRI and that the biological consequences of this event may critically influence the outcome of allergic reactions.

The observations that monocytes/macrophages express IgE receptors (1, 2) and that IgE-mediated ligation of these moieties results in cell activation (3–7) suggest that IgE-dependent activation of monocytes/macrophages plays an important role in the pathogenesis of allergic tissue injury. Low affinity IgE binding sites, i.e., the inducible form of the low affinity IgE receptor CD23 (1) and the IgE-binding protein eBP (2), are the only IgE-binding molecules so far detected on monocytes/macrophages. It was therefore assumed that these cell types preferentially bind preformed IgE complexes rather than monomeric IgE. Based on the observation that cellular binding of monomeric IgE via high affinity acceptor sites is not limited to mast cells and basophils, but also occurs with epidermal Langerhans cells (8, 9), we reevaluated the putatively exclusive role of low affinity IgE binding structures for IgE binding to monocytes. Here we demonstrate that monocytes of atopic individuals can bind monomeric IgE and that this binding occurs via high affinity Fc-IgE receptors similar to those described on basophils and mast cells (10).

Materials and Methods

mAbs Used in This Study. Anti-Leu-4 (CD3), anti-Leu-M3 FITC (CD14), anti-Leu-11a (CD16), anti-Leu-12 (CD19), anti-Leu-19

(CD56; all from Becton Dickinson & Co. [BD],¹ Mountain View, CA); MHM6 (CD23; Dakopatts, Glostrup, Denmark); IV-3 Fab (CDw32), 32.2 F(ab')₂ (CD64; both from Medarex, Inc., West Lebanon, NH); chimeric human IgE (cIgE) and IgG2 (cIgG2) anti-NP (4-hydroxy-3-nitrophenacetyl; both from Serotec, Inc., Oxford, UK); 15-1 and 19-1 (anti-FcεRI, reference 8); and VIAP (anti-alkaline phosphatase, IgG1 control mAb), 7E4 (CD31, reference 11; both provided by Dr. W. Knapp, Institute of Immunology, University of Vienna Medical School) were used. Fab and F(ab')₂ fragments of mAbs were generated using kits from Pierce Chemical Co. (Rockford, IL).

Cell Preparations and Cell Lines. T cell-depleted mononuclear cells (E⁺-MNC) were prepared by density gradient centrifugation of heparinized venous blood followed by depletion of SRBC-binding cells (E⁺-MNC). E⁺-MNC from buffy coats of atopic individuals, i.e., patients suffering from either allergic rhinoconjunctivitis (as defined by clinical criteria, skin prick test, and radioallergen sorbent test) and/or atopic dermatitis (diagnosed according to the criteria cited in reference 12), were subjected to anti-CD3-, anti-CD16-, anti-CD19-, and anti-CD56-immunomagnetic depletion (Dynabeads M-450; Dynal A.S., Oslo, Norway), and monocytes were

¹ Abbreviations used in this paper: AD, atopic dermatitis; BD, Becton Dickinson & Co.; CHO cells, Chinese hamster ovary cells; CHS, allergic contact hypersensitivity; CU, chronic urticaria; MFC, mean fluorescence channel; MNC, mononuclear cells; RC, allergic rhinoconjunctivitis; SA, streptavidin.

purified by centrifuging the remaining cells over a discontinuous Percoll gradient ($d = 1.062$ g/ml). The interface cells regularly consisted of 95–97% CD14^{bright} monocytes and 0.5–2% toluidine blue-positive basophils. Cells denser than 1.062 g/ml consisted of 13–40% basophils. T cells were purified by subjecting E⁺-MNC to hypotonic shock and immunomagnetic depletion of CD19-, CD16-, and/or CD56-positive cells. Chinese hamster ovary (CHO) cells were obtained from the American Type Culture Collection (Rockville, MD). Stable transfectants coexpressing the human FcεRIα- and FcεRIγ-chain (CHOαγ cells) have been described (8).

Immunofluorescence Double Labeling and Flow Cytometric Analysis. To release putative in vivo bound IgE molecules from monocyte surfaces, E⁺-MNC were subjected to acid stripping (0.05 M Tris-HCl, pH 2.5, 1 min on ice) followed by RPMI 1640/10% FCS (both from Gibco Ltd., Paisley, Scotland). 10⁶ E⁺-MNC were exposed to 5 μg/ml anti-Leu-M3 FITC and to either 5 μg/ml biotinylated 15-1, 10 μg/ml biotinylated 15-1 F(ab')₂, 10 μg/ml biotinylated F(ab')₂ fragments of the irrelevant isotype-matched IgG1 control mAb VIAP, or biotinylated cIgE (1, 5, or 25 μg/ml). Cell-bound biotinylated mAbs were visualized by streptavidin-PE (SA-PE, 1 μg/ml; BD). In selected experiments, E⁺-MNCs were incubated for 30 min with graded doses (20, 100, or 500 μg/ml) of either cIgE, cIgG2, or Fc fragments of polyclonal human serum IgG (huIgG Fc; Chemicon International, Inc., Temecula, CA) before exposure to biotinylated 15-1 or 15-1 F(ab')₂. Conversely, E⁺-MNC were incubated with 50 μg/ml of either 15-1 F(ab')₂, MHM6, IV-3 Fab, or 32.2 F(ab')₂ before the reaction with 5 μg/ml biotinylated cIgE. Cellular fluorescence and light scatter parameters were analyzed on a FACScan® (BD). Monocytes were gated simultaneously by their high CD14 expression and by their typical orthogonal light scatter characteristics. The specific reactivity of biotinylated 15-1 F(ab')₂ was expressed as the Δ-mean fluorescence channel number (Δ-MFC: MFC of 15-1 F(ab')₂ reactivity minus MFC of the reactivity of the IgG1 F(ab')₂ control mAb). Alternatively, the specific reactivity of intact mAb 15-1 was calculated by subtracting the MFC of biotinylated 15-1 reactivity in the presence of 500 μg/ml cIgE from the MFC of 15-1 reactivity in the absence of cIgE. The specific binding of biotinylated cIgE was calculated by subtracting the MFC of the medium control from the MFC of cIgE reactivity. Statistical significances were determined using the unpaired Student's *t* test and *p* < 0.05 was considered to be statistically significant.

Cytoplasmic RNA Preparation and Northern Blotting. 5 × 10⁷ purified monocytes, purified T cells, and T cells artificially contaminated with basophil numbers equivalent to those present in the monocyte preparation were solubilized and cytoplasmic RNA was extracted as described (13). Controls included RNA obtained from murine skin, CHO, and CHOαγ cells. 15 μg RNA of each preparation was electrophoresed on a 1.2% agarose/formaldehyde gel and transferred to a GeneScreen™ nylon membrane (New England Nuclear, Boston, MA). Hybridization probes were: (a) FcεRIγ (14), 300-bp Bsp1286I-XbaI cDNA fragment; (b) FcεRIα (15), 900-bp PstI-EcoRI cDNA fragment, and (c) β-actin, 34-meric oligonucleotide (16). cDNA probes were labeled with α-[³²P]-dATP (Amersham International, Buckinghamshire, UK) by random priming, and labeling of the β-actin probe was achieved by adding α-[³²P]dATP to its 3' end using terminal transferase (Boehringer Mannheim Biochemicals, Mannheim, Germany). Prehybridization, hybridization, and washes were performed by standard procedures (17). Blots were exposed to Kodak X-Omat AR films in the presence of intensifying screens at -70°C and were stripped before rehybridization.

Western Blotting. 5 × 10⁷ cells of each population were solu-

bilized in 500 μl Tris-lysis buffer/1% NP-40/0.1% deoxycholic acid (both from Sigma Chemical Co., St. Louis, MO). Equal quantities of lysates (except for CHO and CHOαγ cells) were separated on 10 or 13% SDS-polyacrylamide gels and electroblotted onto Hybond C nitrocellulose membranes (Amersham International). Membranes were blocked in 5% dry milk/0.05% Tween 20 (Sigma Chemical Co.)/PBS, and incubated with either 175 ng/ml mAb 19-1, 175 ng/ml mAb 7E4, or a 1:40,000 dilution of rabbit anti-human FcεRIγ (18). Membrane-bound first-step Abs were reacted with either goat anti-mouse or goat anti-rabbit horseradish peroxidase conjugates (both from Bio-Rad Laboratories, Richmond, CA). Membrane-bound second-step Abs were visualized with ECL® developing solution (Amersham International) and exposed to Kodak X-Omat S films.

Measurement of Cytosolic Ca²⁺ Mobilization. E⁺-MNC were subjected to lactic acid stripping as described (19). 4 × 10⁶ E⁺-MNC in RPMI 1640/10% FCS were allowed to adhere to individual wells of 48-well tissue culture plates (Costar Corp., Cambridge, MA) for 2 h at 37°C. Nonadherent cells were removed by rinsing the wells. 80–90% of the adherent cell population was CD14 positive; no toluidine blue-positive cells were detected. After washing in HBSS (GIBCO BRL, Gaithersburg, MD)/1 mM CaCl₂/0.5 mM MgCl₂ (HBSS/Ca/Mg), cells were loaded with Fluo-3 acetoxymethyl ester and Pluronic F-127 (both from Molecular Probes Inc., Eugene, OR) as described (20). After a 10-min incubation at room temperature, HBSS/Ca/Mg/1% FCS was added for another 40 min. Adherent cells were rinsed with Hepes-buffered medium (137 mM NaCl, 5 mM KCl, 5 mM glucose, 1 mM CaCl₂, 0.5 mM MgCl₂, 10 mM Hepes, 1 mM Na₂HPO₄·12 H₂O, 1 mg/ml BSA; pH 7.3) and were exposed to 20 μg/ml F(ab')₂ or Fab fragments of various mAbs for 20 min at room temperature. After rinsing with Hepes-buffered medium, plates were examined under a confocal laser scanning device (MRC 600; Bio-Rad Laboratories; excitation wavelength, 488 nm) mounted onto an inverted microscope (Axiovert 10; Carl Zeiss, Inc., Oberkochen, Germany). Cell-bound mAb fragments were cross-linked by adding affinity-purified sheep anti-mouse IgG F(ab')₂ (Amersham International) to a final concentration of 10 μg/ml. Cellular fluorescence was periodically measured at an emission wavelength of 515 nm. After monitoring Ab-induced changes, 500 ng/ml ionomycin (Sigma Chemical Co.) was added for maximal induction of the cytosolic [Ca²⁺]. The peak fluorescence intensities of 30 randomly selected single cells were determined and data expressed as fluorescence intensity channel number on a scale ranging from 1 to 255.

Results and Discussion

Peripheral blood monocytes from atopic donors were identified by their high CD14 expression and by their typical cellular granularity (Fig. 1 A). When selected by gating, these cells (five individual donors) showed a homogeneous reactivity with F(ab')₂ fragments of the anti-FcεRIα mAb 15-1 (Fig. 1 C) but did not bind F(ab')₂ fragments of an isotype-matched IgG1 control mAb (Fig. 1 B). The specificity of 15-1 F(ab')₂ binding to monocyte FcεRI was confirmed by the complete inhibition of anti-FcεRIα reactivity after preincubation of the cells with monomeric human IgE (cIgE) but not with monomeric human IgG2 (cIgG2) or human IgG Fc fragments (huIgG Fc; Fig. 1 D). In reverse fashion, FcεRI⁺ monocytes of atopic individuals bound monomeric IgE at low concentrations (Fig. 1 E) whereas FcεRI⁻ monocytes from healthy donors failed to do so (data not shown).

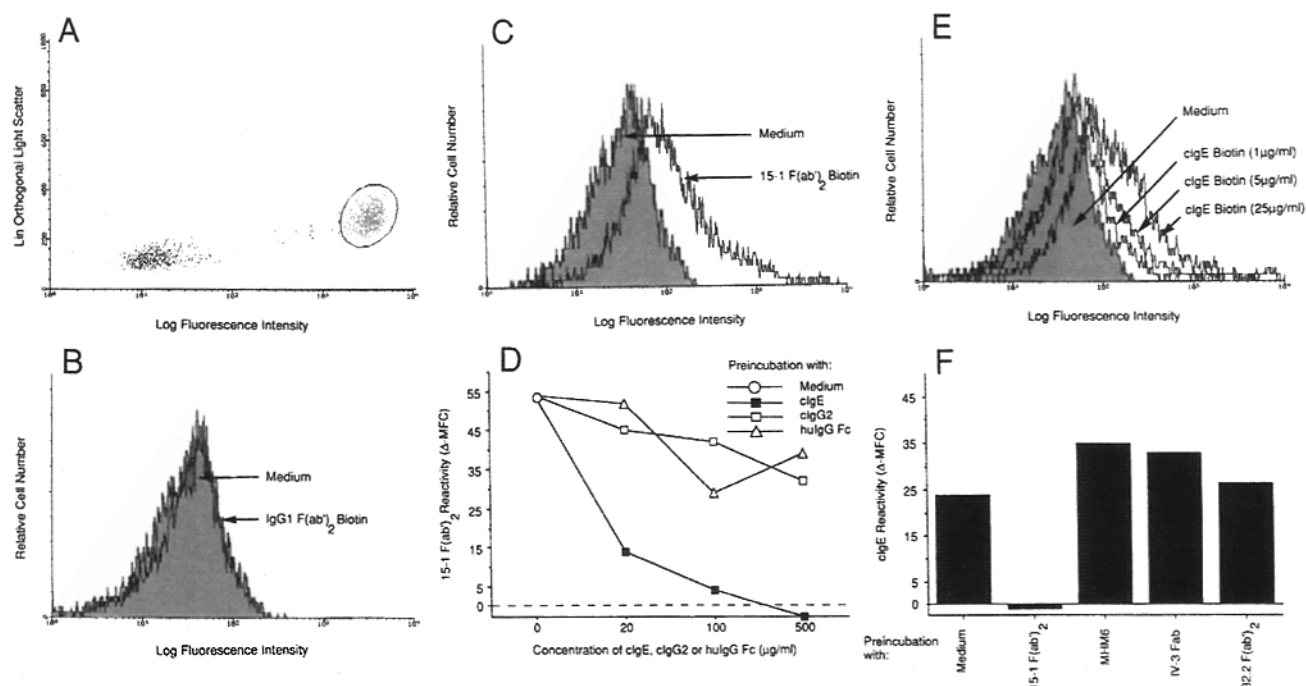


Figure 1. Flow cytometric analysis of monocyte $\text{Fc}\epsilon\text{RI}\alpha$ expression and $\text{Fc}\epsilon\text{RI}\alpha$ -mediated monomeric IgE binding. Representative experiment. (A) Peripheral blood monocytes as identified by intense reactivity with a FITC-labeled anti-CD14 mAb (abscissa) and typical orthogonal light scatter characteristics (ordinate) were gated for the analysis of $\text{Fc}\epsilon\text{RI}\alpha$ expression and IgE-binding capacity. Reactivity of gated monocytes with biotinylated $\text{F}(\text{ab}')_2$ fragments of an IgG1 control mAb (B) or with biotinylated mAb 15-1 $\text{F}(\text{ab}')_2$ (C; open histogram) as visualized by streptavidin (SA)-PE. Closed histograms give the reactivity of SA-PE alone. Logarithmically amplified PE fluorescence is given on the abscissa and the relative cell number on the ordinate. (D) 15-1 $\text{F}(\text{ab}')_2$ reactivity of gated monocytes (ordinate) in the absence (open circle) or in the presence of increasing concentrations (abscissa) of the clgE (closed squares), clgG2 (open squares), or hulgG Fc (open triangles). (E) Reactivity of gated monocytes with graded doses of biotinylated clgE as detected by SA-PE (open histograms) or with SA-PE alone (closed histogram). (F) Binding of biotinylated clgE to monocytes ($\Delta\text{-MFC}$, ordinate) after preincubation of the cells with either medium or 15-1 $\text{F}(\text{ab}')_2$, MHM6 (CD23), IV-3 Fab (CDw32), or 32.2 $\text{F}(\text{ab}')_2$ (CD64).

While these data strongly suggest that monocyte $\text{Fc}\epsilon\text{RI}\alpha$ is responsible for the observed monomeric IgE-binding, a possible contribution of CD23 or even $\text{Fc}\gamma\text{R}$ was not formally excluded. To address this issue we tested a panel of anti-FcR mAbs for IgE-binding inhibition. Selectively, mAb 15-1, directed against the IgE-binding site of $\text{Fc}\epsilon\text{RI}$, completely abolished the binding of monomeric IgE to monocytes (Fig. 1 F). In contrast, mAbs to $\text{Fc}\epsilon\text{RII}$ (MHM6), $\text{Fc}\gamma\text{RI}$ (32.2 $\text{F}(\text{ab}')_2$), or $\text{Fc}\gamma\text{RII}$ (IV-3 Fab) did not interfere with this binding (Fig. 1 F). We therefore conclude that $\text{Fc}\epsilon\text{RI}\alpha$ is expressed on monocytes of atopic individuals and is the only structure capable of binding monomeric IgE on these cells.

To search for a linkage between $\text{Fc}\epsilon\text{RI}$ expression on monocytes and allergic diseases we investigated the occurrence of monocyte $\text{Fc}\epsilon\text{RI}\alpha$ expression in healthy adult volunteers (CO) and in patients suffering from atopic dermatitis (AD), allergic rhinoconjunctivitis (RC), chronic urticaria (CU), and allergic contact hypersensitivity (CHS). We found that specific, i.e., IgE-inhibitable, 15-1 binding to monocytes was significantly ($p < 0.05$) upregulated in atopic individuals (AD, RC) as compared with the nonatopic control groups in which almost no (CO, CHS) or only moderate (CU) $\text{Fc}\epsilon\text{RI}\alpha$ expression was observed (Fig. 2).

To glean information about the molecular composition of the high affinity IgE receptor on monocytes, we screened RNA from "monocytes" (>95% CD14-positive, 1% toluidine blue-positive cells) from two atopic individuals for transcripts encoding the various chains of $\text{Fc}\epsilon\text{RI}$ expressed in basophils and mast cells (10) and detected specific hybridization signals with the $\text{Fc}\epsilon\text{RI}\alpha$ (1.1 kb; Fig. 3 A) and the $\text{Fc}\epsilon\text{RI}\gamma$ (0.8 kb; Fig. 3 B) probes. Since hybridization of basophil-contaminated T cell RNA with the same cDNA probes yielded comparatively much fainter signals, we could exclude the possibility that the $\text{Fc}\epsilon\text{RI}$ transcripts observed in the monocyte RNA preparation originated from contaminating basophils. For unknown reasons, we could not detect $\text{Fc}\epsilon\text{RI}\beta$ mRNA in the monocyte preparations. Apart from procedural considerations, the possibility exists that the high affinity $\text{Fc}\epsilon\text{RI}$ receptor on monocytes consists of α and γ chains only. In fact, this composition reportedly suffices for cell surface expression of the human receptor (21).

Molecular mass characteristics of monocyte $\text{Fc}\epsilon\text{RI}\alpha$ and $\text{Fc}\epsilon\text{RI}\gamma$ were studied by Western blot analysis. Lysates of various cell types were subjected to SDS-PAGE, blotted onto nitrocellulose, and developed with either the anti- $\text{Fc}\epsilon\text{RI}\alpha$ mAb 19-1 (Fig. 3 C, left), the isotype-matched control mAb 7E4

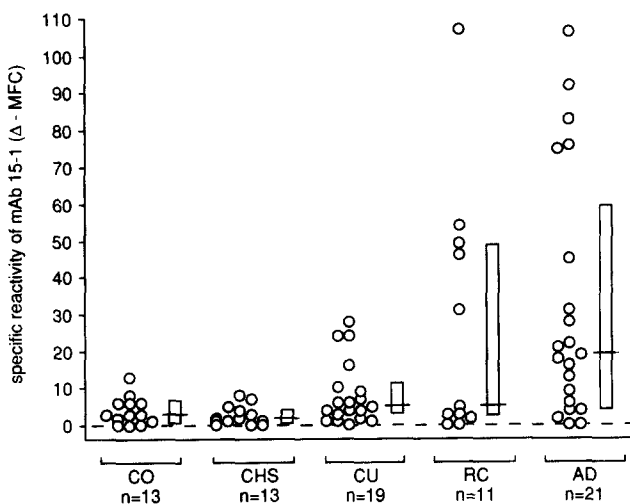


Figure 2. Monocyte FcεRIα expression is significantly upregulated in atopic individuals. FcεRIα expression (*ordinate*) of monocytes from patients suffering from atopic dermatitis (AD), allergic rhinoconjunctivitis (RC), chronic urticaria (CU), allergic contact hypersensitivity (CHS), and healthy adult volunteers (CO) is depicted (*open circles*). Specific mAb 15-1 reactivity of monocytes (Δ-MFC) was calculated as described in Materials and Methods. cIgE-inhibited mAb 15-1 reactivity, i.e., nonspecific reactivity, was comparable in all groups investigated (data not shown). Open bars demonstrate the range from the 25th to the 75th percentile of the distribution of individual FcεRIα expression in each group and horizontal lines indicate the median level of FcεRIα expression of each group.

(Fig. 3 C, *right*), or an antiserum to FcεRIγ (Fig. 3 D). We found identical molecular mass properties for monocyte-, basophil-, and CHOαγ-FcεRIα and FcεRIγ (50–70 and 18 kD, respectively). Control lysates displayed either no (T cells and CHO cells), or only very faint (T cells plus 1% basophils), reactivity with anti-FcεRIα and anti-FcεRIγ reagents. Thus, we conclude that monocytes can express FcεRIα and FcεRIγ chains and that their molecular mass characteristics correspond to those encountered on basophils.

To determine whether monocyte FcεRI expression is of functional significance, we looked for signal transduction occurring after receptor ligation with Fab or F(ab')₂ fragments of mAb 15-1 and FcεRI cross-linking with sheep anti-mouse IgG F(ab')₂. Fig. 4 shows that FcεRI is capable of mediating a sustained cytosolic Ca²⁺ increase in plastic-adherent monocytes quantitatively comparable to that introduced via FcγRII. In contrast, in non-plastic-bound FcεRI⁺ monocytes no, or only minimal, FcεRI-mediated elevation of the cytosolic Ca²⁺ concentration occurred, although these cells vigorously responded to FcγRII cross-linking (data not shown). We therefore conclude that the activation state of monocytes determines the functionality of FcεRI expressed on their surfaces. Thus, it is conceivable that a robust FcεRI-mediated Ca²⁺ response of mononuclear phagocytes *in vivo* depends on their attachment to cellular substrates and/or matrix proteins. Similar activation requirements have been proposed for

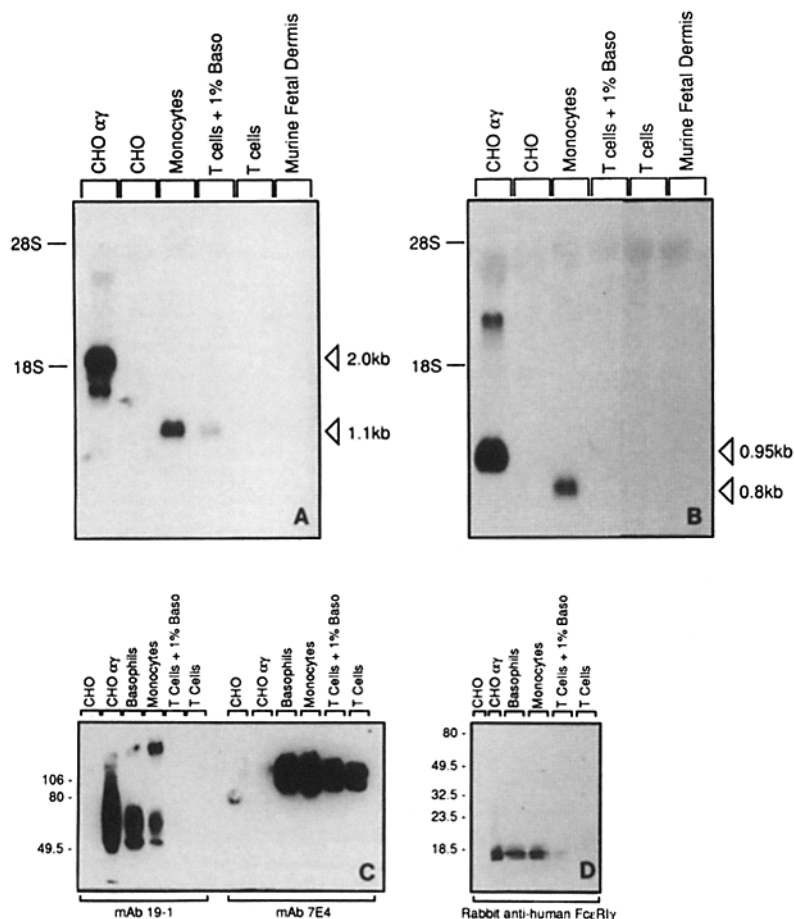


Figure 3. Peripheral blood monocytes of atopic individuals express FcεRIα and FcεRIγ at the mRNA and protein level. Representative experiments. Cytoplasmic RNA from CHOαγ cells, CHO cells, monocytes containing 1% residual basophils, autologous T cells mixed with 1% basophils, pure T cells, and murine skin was hybridized to ³²P-labeled FcεRIα (A) or FcεRIγ (B) cDNA probes. Hybridization to a β-actin probe confirmed that comparable amounts of RNA had been loaded in all lanes (data not shown). (C) Cell lysates of CHO cells, CHOαγ cells, basophils, monocytes containing 1% residual basophils, T cells mixed with 1% basophils, and pure T cells were size fractionated by SDS-PAGE and analyzed by Western blotting for FcεRIα expression using mAb 19-1 (*left*). For control purposes, CD31 expression was determined using the isotype-matched mAb 7E4 (*right*). The high molecular mass band in the monocyte lane corresponds to residual mouse mAbs from the purification procedure. Molecular mass calibration is shown on the left in kilodaltons. (D) Reactivity of the cell lysates with rabbit anti-human FcεRIγ. Normal rabbit serum yielded no reactivity (data not shown).

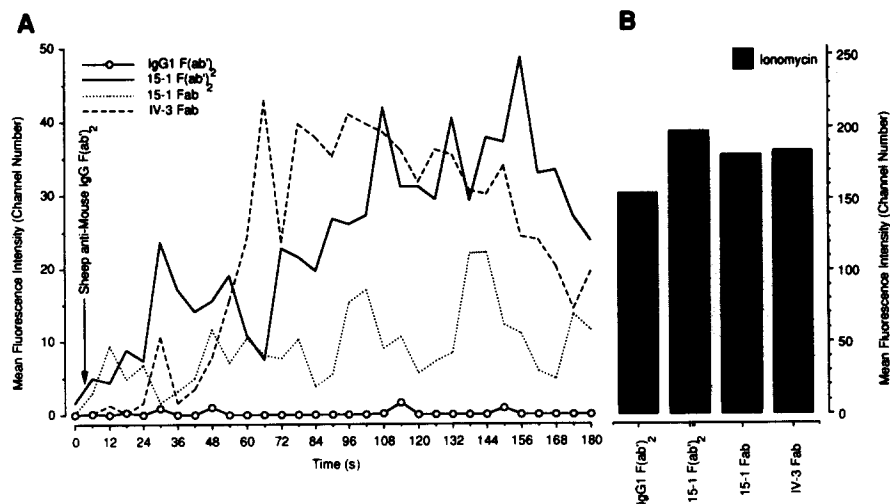


Figure 4. Elevation of cytosolic free Ca^{2+} after cross-linking of monocyte $\text{Fc}\epsilon\text{RI}$. Representative experiment. (A) Fluo-3 loaded, plastic-adherent E^- -MNC were reacted with either 15-1 F(ab')_2 (solid line), 15-1 Fab (dotted line), IV-3 Fab (dashed line), or with an IgG1 F(ab')_2 control mAb (solid line/open circles). Cell-bound mAbs were cross-linked with sheep anti-mouse IgG F(ab')_2 at the indicated time point. Cellular Fluo-3 fluorescence, as a relative measure of the cytosolic Ca^{2+} concentration, was monitored in 6-s intervals over a total time of 180 s by laser video microscopy. The mean fluorescence intensity channel number (ordinate) of 30 individual cells for each investigated time point was plotted vs. the elapsed time (abscissa). (B) At the end of the monitoring period, ionomycin was added to each reaction in order to maximally increase intracellular Ca^{2+} concentrations. The mean fluorescence intensity (ordinate) of the same 30 cells monitored in A is displayed.

rat basophilic leukemia cells in which the adherence-dependent activation of the focal adhesion kinase p125^{FAK} amplifies signal transduction via $\text{Fc}\epsilon\text{RI}$ (22).

The IgE-mediated allergic reaction has so far been solely attributed to biological events initiated by the interaction of multivalent allergens with mast cell/basophil-bound IgE molecules (23, 24). The recent discovery of $\text{Fc}\epsilon\text{RI}$ on Langerhans cells (8, 9) and the demonstration of functionally active $\text{Fc}\epsilon\text{RI}$ on adherent mononuclear phagocytes (this study) possibly add a new facet to our understanding of the pathogenesis of IgE-mediated allergic reactions. In atopic individuals, the allergic reaction is often composed of an immediate wheal and flare response and a chronic late phase reaction (24). Our results suggest: (a) that not only mast cells/basophils but

also mononuclear phagocytes of atopic individuals carry $\text{Fc}\epsilon\text{RI}$ -bound monomeric IgE molecules; and (b) that, in addition to mast cells/basophils, IgE-bearing, $\text{Fc}\epsilon\text{RI}^+$ monocyte-derived tissue macrophages become directly activated after the interaction with multivalent allergens. This may result in enhanced allergen presentation by these cells and/or in the synthesis and release of proinflammatory cytokines (3), eicosanoids (4, 5), enzymes (6), or oxygen radicals (7) in a pattern not necessarily identical to that seen with mast cells and basophils. Thus, the interaction of allergen with $\text{Fc}\epsilon\text{RI}^+$ -bound allergen-specific IgE on macrophages may critically influence the duration, quality, and quantity of allergic reactions.

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