

Function and regulation of FcεRI expression on monocytes from non-atopic donors

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Summary

Background The high affinity receptor for IgE (FcεRI) has recently been identified on antigen presenting cells, i.e. Langerhans cells and monocytes from atopic donors and it was hypothesized that FcεRI expression levels correlated with allergy.

Objective The aims of the study was to investigate the function and expression of FcεRI on monocytes from non-atopic donors.

Methods Purified monocytes or peripheral blood mononuclear cells were used to study FcεRI expression and signal transduction on CD14 positive cells by flow cytometry and/or confocal laser microscopy.

Results Freshly isolated monocytes from healthy individuals ($n = 58$) were shown to express FcεRI (median 18%, range 2–66%). No IgE was bound to these receptors *in vivo*, and *in vitro* no significant binding of complete IgE molecules could be obtained. IgE positive monocytes from atopic donors were also found to have free FcεRI, incapable of binding IgE *in vitro*. On all CD14 positive cells free FcεRI expression was rapidly and completely lost during culture in conventional culture media (IMDM, RPMI) but not in phosphate buffered saline (PBS). Moreover, signal transduction through free FcεRI appeared to be inhibited. However, both IgE binding and calcium mobilization were restored by treatment of fresh non-atopic monocytes with neuraminidase. Importantly, culturing these monocytes overnight in conventional medium containing 2 µg/mL IgE induced a cycloheximide insensitive accumulation of IgE bound to FcεRI and, in addition, led to cell activation.

Conclusion Monocytes from both atopic donors and healthy individuals express FcεRI, but the previously reported different expression levels between the two groups seem to be directly related to the absence or presence of IgE in the serum. This may be due to the fact that FcεRI is subjected to a constant turnover process which is slowed down but not prevented by ligand binding. In addition, free FcεRI on non-atopic monocytes are under control of a neuraminidase sensitive structure(s), which influences signal transduction and IgE binding.

Keywords: allergy, FcεRI, IgE, monocyte activation, regulation

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Introduction

The interaction of immunoglobulin E (IgE) with its receptors may play an important role as a defence system against parasites [1]; less beneficial however, is the role of IgE and

IgE-binding structures in human allergic conditions, which was established as early as 1921 [2]. In 1966, IgE was found to be the main component involved in allergic conditions and the counterstructures to be present on mast cells and basophils [3]. Today, with the improvement of detection methods and the availability of monoclonal antibodies, three IgE-binding structures have been characterized: the

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high affinity receptor Fc ϵ RI [4–6], the low affinity receptor (Fc ϵ RII, CD23) and galectin-3, the epsilon binding protein (ϵ BP), which is a β -galactoside binding lectin and binds IgE with low affinity [7,8]. Until recently, the high affinity receptor Fc ϵ RI, was found only on mast cells and basophils [9] where aggregation of Fc ϵ RI initiates several biochemical events leading to degranulation and release of histamine as well as other inflammatory mediators [10]. The availability of monoclonal antibodies (MoAbs) specific for the α -chain of the human high affinity receptor [5,11] has facilitated the detection of Fc ϵ RI on several other cells, such as Langerhans' cells [11–13], eosinophils [14] and monocytes from atopic donors [15]. The presence of both Fc ϵ RI and CD23 on antigen presenting cells has led to a new role for IgE in immune responses [reviewed in 16,17]. IgE bound to antigen presenting cells dramatically enhances their capacity to initiate an immune response [18].

Maurer *et al.* [15] recently reported that, Fc ϵ RI could be found at high levels on monocytes from atopic donors, whereas monocytes from normal healthy individuals showed no or only moderate expression. In contrast to this study, we report here that monocytes from all healthy donors ($n=58$) express substantial levels of Fc ϵ RI and that the receptor expression is influenced by the isolation procedure. Further, we describe mechanisms of receptor regulation and function on monocytes from normal and atopic donors and present indications that Fc ϵ RI can be subjected to a recycling mechanism.

Materials and methods

Reagents and antibodies

Fluo-3-acetoxymethylester was purchased from Molecular Probes (Eugene, OR), 7-aminoactinomycin D (7-AAD) and cycloheximide from Sigma (St Louis, MO, USA).

IgG1 isotype control, anti-IgG1-phycoerythrin (-PE) and anti-IgG2a-PE goat anti-mouse (GaM) were purchased from Southern Biotechnology (Birmingham, AL, USA), mouse IgG1-FITC isotype control, Streptavidin-PE from Becton Dickinson (San Jose, CA, USA), anti-human CD14-FITC and CD64 (both IgG1) from Serotec (Oxford, UK), CD3-FITC, CD16-FITC, CD23-FITC [19] (all IgG1) and CD32 (IgG2a) from Immunotech (Marseille, France), CD54 (ICAM-1) from Bender (Vienna, Austria), CD19-FITC (IgG1) and swine-anti-rabbit-FITC from DAKO (Glostrup, DK), mouse IgM and IgG2a isotype control from Coulter (Hialeah, FL, USA), goat-anti-mouse-PE (GaM-PE) from Tago (Burlingame, CA, USA), rabbit-anti-human galectin-3 from Dr Liu (Scripps, CA), CsLeX1-hybridoma was purchased from ATCC and is of IgM-isotype.

Anti-human Fc ϵ RI- α chain MoAbs: 15.1 hybridoma (competitive for IgE-binding, isotype IgG1) and 19.1

MoAb (ascites, IgG2a) were kind gifts from Dr J.-P. Kinet (National Institutes of Health, Rockville, MD, USA) [11], 6F7 and 22E7 (both IgG1) were kind gifts from Dr R. Chizzonite (Hoffmann-La Roche, Nutley, NJ, USA) [5]; Hapten-specific 4-hydroxy-3-nitrophenacetyl (NIP) chimaeric IgE antibody (clone JW8/1) was purchased from Serotec (Oxford, UK), human myeloma IgE from Chemicon (Temecula, CA, USA), mouse IgE from Sigma (Deisenhofen, Germany) and BioMakor BM (Rehovot, Israel).

Recombinant IL-4 was provided by Dr E. Liehl (Sandoz, Vienna, Austria), the 96-well plates were purchased from Costar (Fernwald, Germany) and the Micronic[®] tubes from Micronic systems (Lelystad, the Netherlands).

RPMI, IMDM both GIBCO BRL (Gaithersburg, MD, USA) were supplemented with 100 mM L-glutamine, 5000 U/mL penicillin, 5000 μ g/mL streptomycin and either 1% pooled human AB rhesus positive serum (ABS), 1% or 10% FCS. PBS def. was supplemented with 100 mM L-glutamine, 5000 U/mL penicillin, 5000 μ g/mL streptomycin and 1% ABS. All media were tested negative for LPS contamination.

Cells

Healthy (non-atopic) volunteers ($n=58$) classified for blood transfusion and did not show signs of infection. All donors filled out a questionnaire dealing with a possible history of atopy, only donors who scored negative on all questions were considered non-atopic. Atopic donors ($n=4$) were patients with atopic dermatitis diagnosed according to the criteria by Hanifin and Rajka [20].

PBMCs were isolated by density centrifugation on Ficoll-Separating Solution at 20°C, 1500 *g* for 20 min (Biochrom, Berlin, Germany), the interphase was collected, washed twice in PBS def. at 4°C and kept on ice until use.

Primary human monocytes were obtained by elutriation as previously described [21]. Briefly, monocytic cells were separated from the buffy coats of a single blood unit by centrifugation through a 0.8 mM sodium citrate-containing Lymphopaque gradient. The cells obtained ($0.5-1 \times 10^9$) after elimination of residual platelets, were resuspended in PBS def. containing 0.27 mM EDTA 1% BSA. Elutriation was performed at 10°C in this medium using a JE-6 rotor in a J-6 M centrifuge (Beckman Instruments, CA, USA) and stepwise reduction of the centrifugal force and increase of the medium flow.

Flow cytometric analysis of cell surface receptors

Primary human monocytes or mononuclear cells fresh or after culture, were washed twice in PBS def. containing 1% FCS. After culture a rubber policeman was used to detach any adherent monocytes from all culture bottles. The cells

(1×10^6 c/mL) were preincubated for 30 min at 4°C in PBS def. containing 1% FCS and 10% ABS or 5% ABS plus 5% normal mouse serum (NMS). ABS and NMS were added to block unspecific binding of antibodies to human FcR. Aliquots (100 µL) of cells were added to the first antibody into a 96-well U-bottom plate and incubated for 30 min at 4°C. After incubation cells were washed three times with 250 µL PBS def. 1% (fetal calf serum) FCS. Samples with directly labelled antibodies were then resuspended in fixing buffer (PBS def., 1% paraformaldehyde, 0.1% natrium azide) and transferred to Micronic® tubes. Samples with unlabelled antibodies were resuspended in 100 µL PBS def. 1% FCS 10% ABS and the second step antibody added. The same procedure was applied for all staining steps. Negative controls of the same isotype were included to rule out non-specific staining. A viability measurement with 7-aminoactinomycin D was performed to exclude dead cells. Analysis by flow cytometry used a FACScan (Becton Dickinson, San Jose, CA, USA) equipped with 'Lysis II' version 1.0 software. Percentage positivity is given as the percentage above the isotype control, the fluorescence intensity is indicated as MFI representing the fluorescence intensity of the sample minus the intensity of the relevant control.

Detection of FcεRI α-chain by Western blot

Magnetic cell sorting (MACS) was performed according to the manufacturer's instructions, using CD14-beads (all by Miltenyi Biotec Inc., Sunnyvale, CA, USA) followed by a Western blot analysis on the CD14 positive fraction, as previously described [22] using the 19.1 ascites in 1/200 dilution. A control was performed by incubating the membrane with GaM-peroxidase alone.

Neuraminidase treatment

Cells were suspended in PBS def. at 5×10^6 cells/mL, mixed with 0.3 U/mL Test-Neuraminidase (Behring, Marburg, Germany) and incubated for 30 min at 37°C. The cells were then washed twice in PBS def. 1% FCS and used in the experiments. Efficiency of neuraminidase treatment was assessed by staining for Sialyl Lewis x (MoAb CsLeX-1) before and after.

Calcium mobilization measured with a confocal laser scanning device

Elutriated monocytes untreated or treated with neuraminidase, resuspended at 10^6 /mL in PBS 20% FCS, were allowed to adhere to individual slide chambers (Costar) for 2 h at 37°C. Non adherent cells were removed by rinsing the wells with HBSS (GIBCO BRL) containing 1 mM CaCl₂

and 0.5 mM MgCl₂ (HBSS/Ca/Mg). After 20 min with 3 µM Fluo-3 (Molecular Probes) to load the cells, HBSS/Ca/Mg was added for another 40 min. Adherent cells were rinsed with HBSS/Ca/Mg before activation and stored at 4°C until use. Before activation, cells were warmed to 37°C for 5 min. Chambers were examined under a confocal laser scanning device (MRC 600, BioRad Laboratory, excitation wavelength, 488 nm) mounted onto an inverted microscope (Axiovert 10; Carl Zeiss Inc.). Cellular fluorescence was measured every 8 s at an emission wavelength of 515 nm.

Results

The high affinity receptor for IgE (FcεRI) is present on monocytes from non-atopic donors

We have tested elutriated monocytes and peripheral blood mononuclear cells (PBMCs) from 58 non-atopic individuals. FcεRI was found to be present on the monocytes from all donors, a typical staining pattern is shown in Fig. 1 (a). Expression was detected by flow cytometry using MoAb 15.1 F(ab)₂-biotin on cells gated for CD14 expression and verified by staining with three other MoAbs detecting different epitopes on FcεRIα (19.1 [11], 6F7 and 22E7 [5]), all with comparable results. The data were confirmed by Western blot analysis after isolation of CD14 positive cells (Fig. 1b) and demonstrate the presence of FcεRI on normal monocytes. The results are summarized in Fig. 1(c), with FcεRI expression ranging from 2% to a maximum of 66% with a median of 18%.

Regulation of FcεRI expression

As our initial findings diverged from published data [15], we analysed which variations in isolation and in staining techniques could account for the observed differences. We found that, in agreement with unpublished observations on Langerhans' cells, overnight culture in media at 37°C led to a disappearance of FcεRI receptor expression on CD14 positive elutriated monocytes or PBMCs (Fig. 2 a and b). This downregulation was independent of the serum content of the medium or shaking overnight to prevent adherence (data not shown). The decrease of receptor expression was already visible after a culture period of 1 h and a reduction to less than half of the initial level could be observed after 3 h. Only minimal receptor expression remained detectable after 6 h (Fig. 2 a). These results indicate that long isolation procedures, the use of conventional culture media and temperatures above 4°C negatively influenced FcεRI expression and subsequent functional assays.

As a prerequisite to conducting experiments investigating signal transduction and receptor regulation, we were prompted to search for a way of prolonging the time of

stable receptor expression. We found that suspending the cells in PBS 1% ABS stabilized FcεRI expression for more than 15 h at 37°C and led to a selective increase in receptor expression (Fig. 2c and d) without influencing other surface receptors, such as CD14 or CD64 (data not shown). Furthermore, the increase in FcεRI expression is time dependent, usually maximal after 6 h (Fig. 2c). FcεRI was still present after 40 h in PBS at 37°C but cell viability was reduced without additional nutrients (data not shown).

To exclude the influence of other mononuclear cell types on receptor expression, we compared elutriated monocytes with peripheral blood mononuclear cells (PBMCs) from the same donor, and found the percentage of expression to be identical both immediately after isolation and after overnight culture of the cells. Experiments with blood cells cultured without prior ficoll-separation in medium or PBS also gave matching results (data not shown). This demonstrated, that the regulation of FcεRI expression was independent of the presence of non-monocytic cells and was not influenced by the elutriation procedure. Viability after a 15 h culture in PBS was comparable to IMDM cultures, as was the recovery of cells.

Responsiveness to IL-4

Interleukin-4 (IL-4) has been shown to be one of the major cytokines upregulated in allergic disease [23,24]. It has been reported to induce CD23 expression on many cell types including B cells [25], monocytes [26,27] and Langerhans

cells [28], a cell population, which also was reported to carry FcεRI [11,12]. We therefore studied the influence of IL-4 on normal monocytes in overnight culture. We found that IL-4 (50 U/mL) induced CD23 expression in PBS 1% ABS as well as in IMDM 1% ABS. However, IL-4 had no effect on FcεRI expression, which, as described above, was lost in IMDM but not PBS (Table 1).

FcεRI on normal monocytes does not bind IgE

Monocytes from healthy individuals express FcεRI, but carry no detectable *in vivo* bound IgE (data not shown). In addition CD23 was negative on all donors tested. Unexpectedly, our attempts to bind IgE *in vitro* to freshly isolated monocytes were not successful. Even if minor amounts of IgE were bound *in vitro*, no correlation with the level of FcεRI expression was found (Fig. 3 a).

Atopic blood

The above experiments were also performed with PBMCs from atopic donors ($n = 4$) to define similarities and differences. After PBMC isolation, large amounts of *in vivo* bound IgE were detected on the monocyte surface, but there was also free FcεRI, to which no additional IgE could be bound *in vitro* (Table 2). Subsequently, the cells were cultured overnight in PBS 1% ABS or IMDM 1% ABS as described above, without removing the *in vivo* bound IgE. After 15 h the same pattern of FcεRI expression was

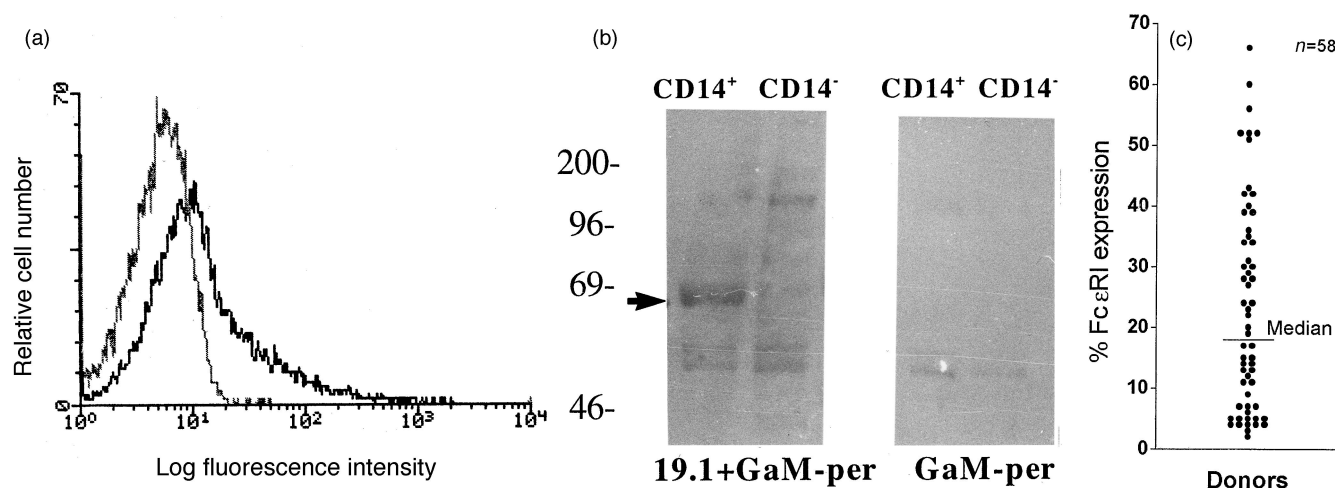


Fig. 1. FcεRI is present on the membrane of monocytes from non-atopic donors. (a) represents a typical staining on freshly isolated cells from a non-atopic individual. The monocytes were identified and gated by their morphological characteristics and their positivity for CD14. Reactivity with MoAb 15.1 F(ab)₂-biotin was determined. Per cent positivity (28.2%) and mean fluorescence intensity (MFI = 7.5) are given as the difference between sample and control. (b) shows the Western blot on the CD14 positive fraction. Cell lysates of elutriated CD14 positive monocytes from a single donor were size fractionated by SDS-PAGE and analysed for FcεRI-α expression using 19.1 MoAb. As control the membrane was blotted with GaM-peroxidase. Molecular mass calibration is shown on the left in kDa. (c) illustrates the individual expression levels of FcεRI on freshly isolated non-atopic monocytes ($n = 58$). The median level of expression was 18% (range 2–66%).

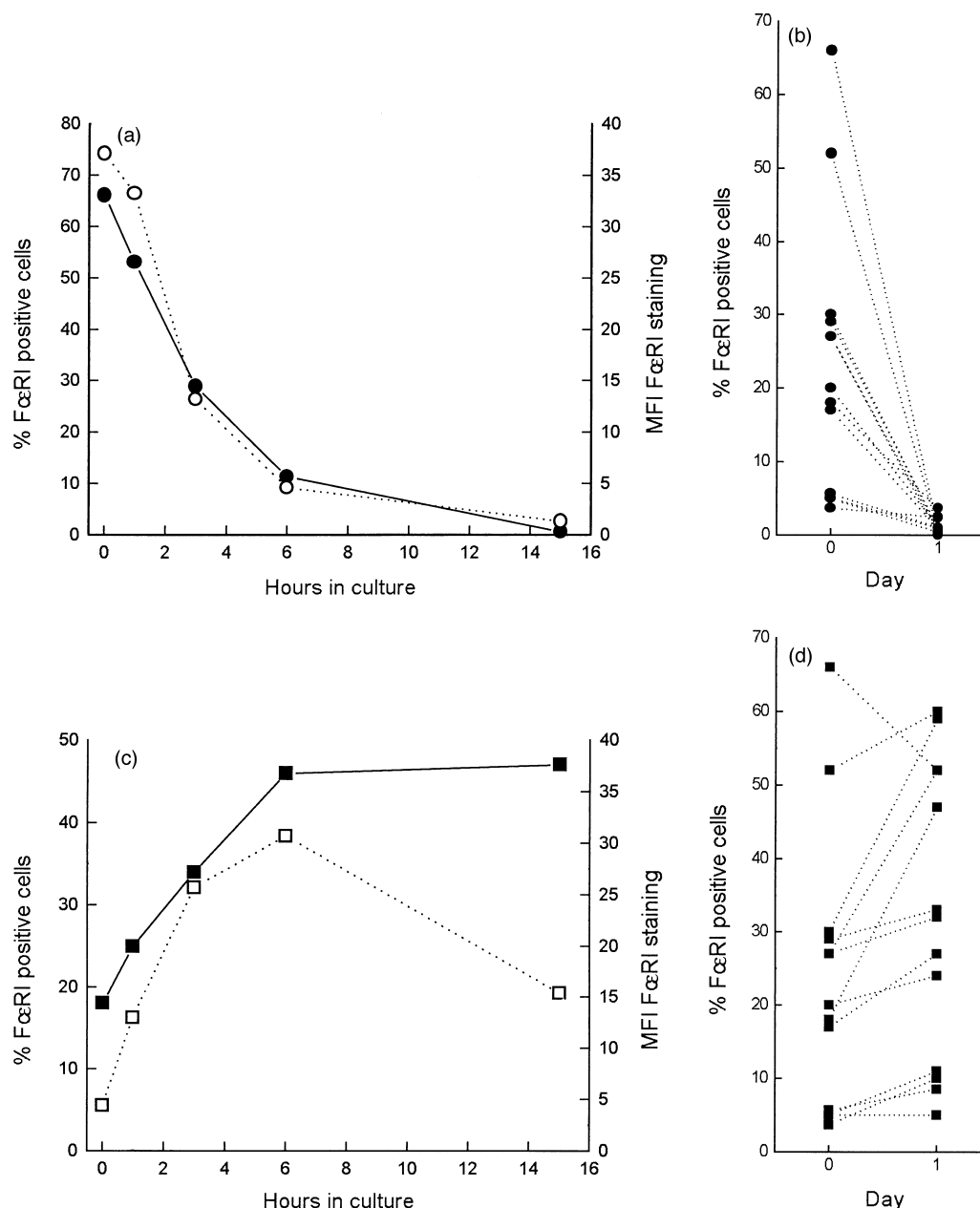


Fig. 2. FcεRI expression and regulation. The four panels illustrate the kinetics of FcεRI expression on monocytes from non-atopic individuals. The cells were cultured for the times indicated, stained with CD14-F (1 : 40), 15.1 F(ab)₂-biotin (10 µg/mL) and streptavidin-PE (1 : 20) and measured by gating for CD14 positive cells only. (a) Downregulation of FcεRI expression in medium (IMDM 1% ABS) with time on monocytes from a single donor. (b) FcεRI positivity on monocytes from 13 donors cultured overnight (15 h) in IMDM. The downregulation is significant ($p(T \leq t) = 0.001$; two tailed paired student *t*-test). (c) Time-dependent upregulation of FcεRI expression in PBS (deficient of Ca⁺⁺ and Mg⁺⁺ ions) 1%ABS on monocytes from a single donor. (d) FcεRI positivity on monocytes from the same 13 donors described in Fig. 2b after culture for 15 h in PBS. The upregulation is again significant ($p(T \leq t) = 0.022$; two tailed paired student *t*-test).

observed as on non-atopic monocytes, i.e. the disappearance of free receptors in IMDM and an increase in PBS. In addition, the amount of *in vivo* cell-bound IgE was strongly reduced in IMDM but only to a minor extent in PBS

(Table 2). This indicated, that FcεRI was subjected to the same regulatory mechanisms on monocytes from healthy and atopic donors. Moreover, these results suggested that FcεRI on monocytes is internalized without cross-linking

and that IgE binding slows down the internalization process. This would be in contrast with what has been described for mast cells and basophils.

Increased FcεRI expression on monocytes due to interaction with ligands

To test the hypothesis that FcεRI is subjected to a turnover process, monocytes from a non-atopic donor were cultured in an 'atopic' environment by adding IgE (2 µg/mL). After overnight culture in IMDM IgE accumulated on the monocyte cell surface (Fig. 4 a). The same effect was observed after culture in PBS, but additional unoccupied FcεRI was

detectable (data not shown). Overnight incubation at 4°C prevented both the downregulation of FcεRI as well as the accumulation of IgE. This excludes the possibility that the lack of IgE binding was due to a decreased affinity as reported by Pecoud *et al.* [29]. Inclusion of complete MoAb 15.1, F(ab)₂ or F(ab) fragments, but not anti-CD23 MoAb, in the same experimental design also led to accumulation of FcεRI on the cell surface (Fig. 4b).

To determine whether the IgE accumulation was due to *de novo* synthesis of FcεRI, the experiment was repeated in the presence of cycloheximide (1 µg/mL), a protein synthesis inhibitor [30,31]. While the IL-4 induced CD23 expression and consequent binding of IgE to CD23 were

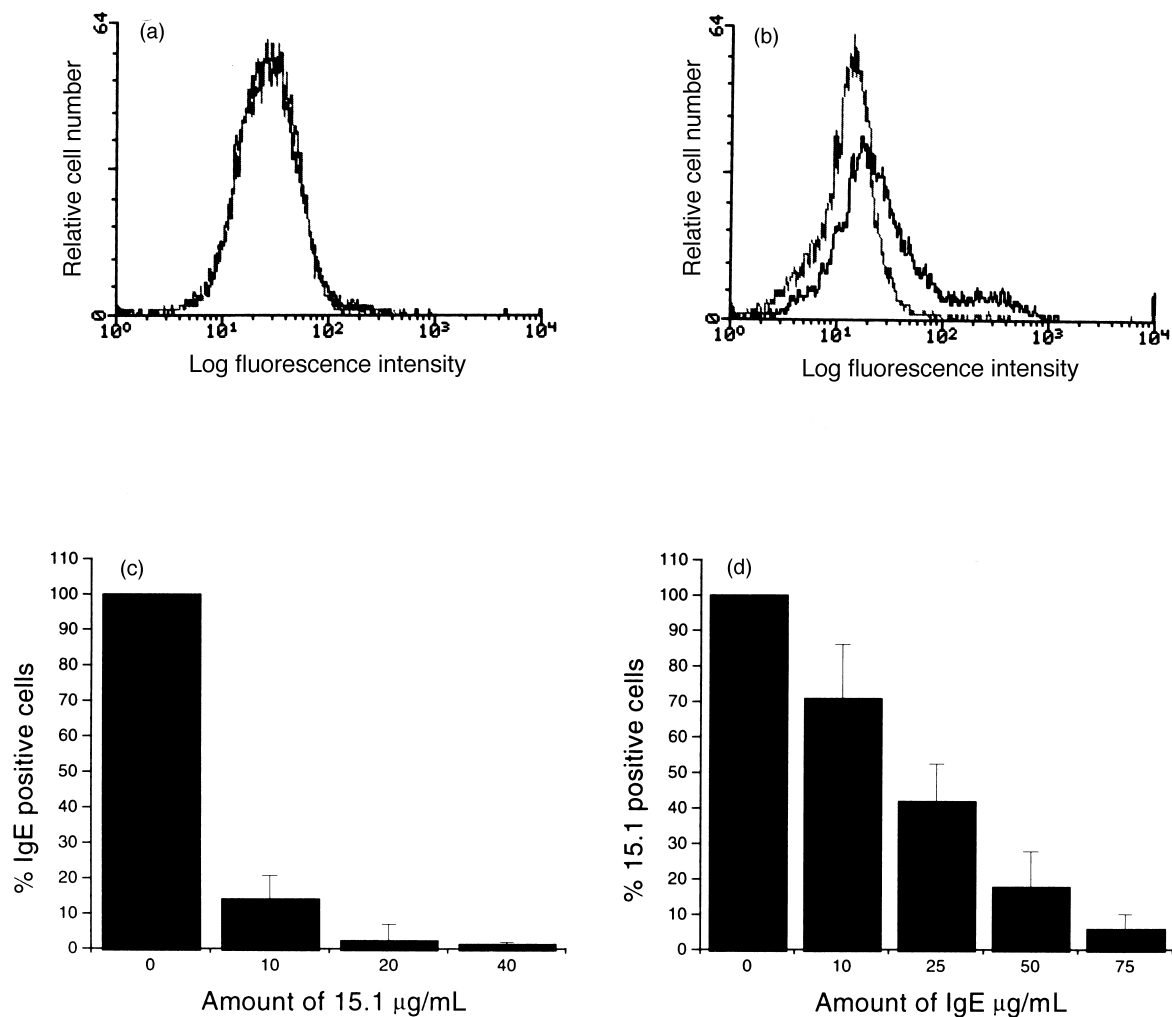


Fig. 3. Inhibition of IgE binding with MoAb 15.1 and *vice versa* on neuraminidase treated cells from non-atopic individuals. (a) Flow cytometric analysis of freshly isolated monocytes shows no binding of complete human IgE-biotin (5 µg/mL) detected with streptavidin-PE, 0.7% positive, MFI = 0.2). (b) After neuraminidase treatment (0.3 U/mL) IgE binding is detectable on the same cells (23.7%, MFI = 15.5). (c) Monocytes from six donors after neuraminidase treatment showing the reduction of IgE positivity (5 µg/mL) in the presence of increasing amounts of 15.1. Data are expressed as mean ± SD and for comparison the individual values were each set at 100%. (d) Monocytes from seven donors after neuraminidase treatment illustrating the dose-dependent reduction of FcεRI positivity (10 µg/mL 15.1 F(ab)₂-biotin/streptavidin-PE) with increasing amounts of IgE, set at 100% and expressed as mean ± SD.

Table 1. IL-4 induced CD23 expression on non-atopic monocytes

Condition	CD23		FcεRI		In vitro bound IgE	
	%	MFI	%	MFI	%	MFI
After isolation	0.1	0.2	29.9	7.5	0.7	3.7
15h IMDM	0.3	0.3	1.0	0.4	0.3	0.5
15h IMDM + IL-4	25.8	5.4	1.6	1.3	13.2	5.2
15h PBS	3.4	0.7	43.2	13.0	2.0	1.8
15h PBS + IL-4	67.4	11.1	48.4	13.0	28.8	10.0

Elutriated monocytes from a single donor were stained immediately after isolation and after overnight culture in either PBS def. 1% ABS or IMDM 1% ABS with or without 50 U/mL of IL-4. The results are given as percentage of positivity (%) and as MFI.

abolished by cycloheximide (Table 3), no alteration of FcεRI bound IgE accumulation was observed (Fig. 4a and Table 3).

Adding IgE or MoAb 15.1 led to cluster formation after overnight culture, which prompted us to investigate the expression of the adhesion molecule CD54 (ICAM-1) in the varying culture conditions. ICAM-1 was expressed at 100% in all conditions, but the intensity of expression proved to be indicative of the general activation state of the monocytes. While the addition of IL-4 to the medium did not lead to increased intensity of ICAM-1 expression, IgE, MoAb 15.1 F(ab)₂ and F(ab) had a pronounced effect on the receptor. ICAM-1 induction was prevented by adding cycloheximide to the media (Table 3) [30].

IgE binding to FcεRI on non-atopic human monocytes is influenced by sialyl-groups. We consequently looked for factors, with a reported influence on the binding of IgE to its receptor, especially since we noticed that, biotinylated Fcε fragments bound to FcεRI. This binding could be blocked by MoAb 15.1 (data not shown). One of the first targets was galectin-3 (εBP) which may affect binding of IgE to the high affinity receptor on human Langerhans' cells [7]. Galectin-3

can be eluted from the cell surface by incubation with lactose [7], but this treatment did not alter IgE binding to the monocytes or expression of FcεRI. Furthermore, no binding was observed with a specific rabbit anti-human galectin-3 MoAb, indicating a complete absence of this structure on freshly isolated human monocytes (data not shown).

Previous reports indicated that sialyl groups on the cell membrane or on the receptor itself sterically hinder and diminish FcεRI function [29,32]. We therefore treated the monocytes with neuraminidase and, whilst neither human nor mouse IgE bound significantly before treatment, positivity was observed after neuraminidase treatment (Fig. 3a and b). The specificity of the binding was illustrated by the dose-dependent inhibition of IgE binding by 15.1 (Fig. 3c) and *vice versa* 15.1 positivity by IgE (Fig. 3d). The expression of FcεRI or CD14 on freshly isolated monocytes was not affected by the neuraminidase treatment (data not shown). The disappearance of sialyl Lewis x (sLeX), detected by the specific MoAb csLeX1, was taken as an efficiency-indicator of the neuraminidase treatment. The binding of Fcε fragments and the neuraminidase treatment show, that the binding site for IgE on the α-chain of FcεRI is func-

Table 2. Receptor kinetics on atopic blood

Condition	In vivo bound IgE		Free FcεRI		In vitro bound IgE		CD23	
	%	MFI	%	MFI	%	MFI	%	MFI
After isolation	79.5	33.8	16.2	3.9	1.7	0.5	0.1	0.1
15h IMDM	27.4	16.5	4.0	1.6	2.1	1.2	0.0	0.0
15h PBS	64.9	23.0	47.4	15.1	19.9	5.0	0.0	0.0

PBMCs from an atopic donor were isolated by ficoll separation, stained for the various receptors and cultured overnight in either PBS def. 1% ABS or IMDM 1% ABS. All stainings were measured with a morphological gate and, with the exception of CD23, a CD14 gate. The results are given as percentage of positivity (%) and as MFI.

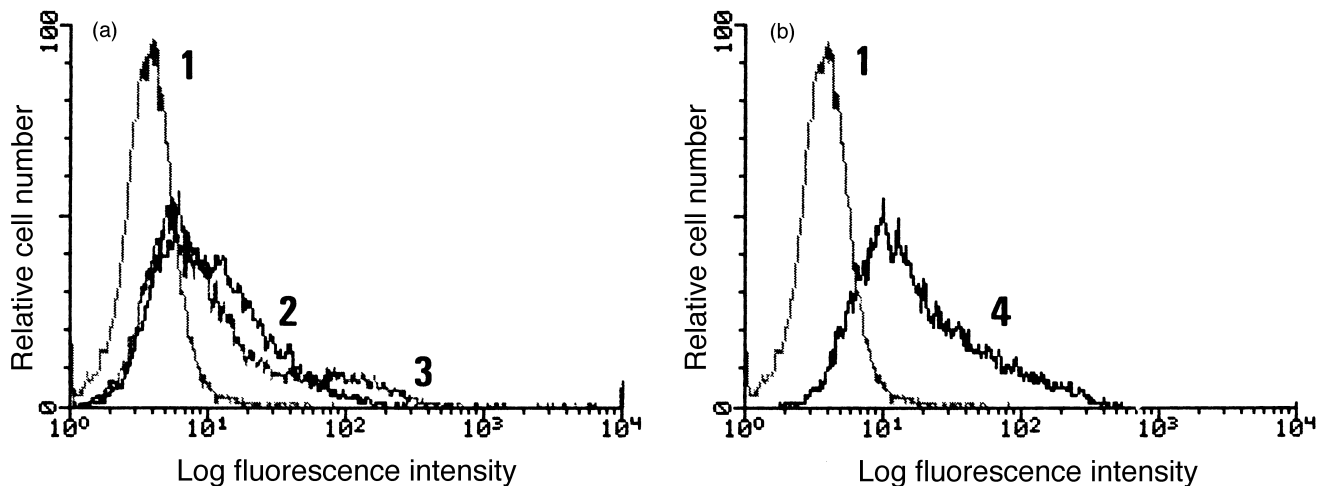


Fig. 4. FcεRI accumulation experiments. The monocytes from a non-atopic donor were cultured overnight (15 h) in IMDM 1% ABS and in the presence of either IgE (2 µg/mL), IgE plus cycloheximide (10 µg/mL) or MoAb 15.1 (10 µg/mL). Positivity of this donor for FcεRI on day 0 is depicted in Fig. 1a. (a) Accumulation of IgE. Line 1 represents the control, line 2 the accumulation with IgE (43.5%, MFI = 10.5), which cannot be prevented by the presence of cycloheximide in the culture medium (line 3, 36.7%, MFI = 11.1). (b) shows the accumulation achieved overnight in the presence of MoAb 15.1 (line 4, 62.1%, MFI = 17.9) with line 1 again representing the control staining.

tional, but that the binding of intact IgE molecules is prevented by steric hindrance of sialyl groups. We assume that these groups are present on secondary molecules [32,33], rather than on the receptor itself [29]. CD23 was expressed at less than 2% on all donors.

Signal transduction via FcεRI on freshly isolated monocytes

Not only was the binding of IgE to FcεRI on freshly isolated monocytes from healthy individuals minimal, but in addition no signal transduction measured by calcium mobiliza-

tion after cross-linking was observed independent whether the cell were allowed to adhere or not (Figs 5a–d). This stands in contrast to previously published results on monocytes from atopic donors, where signal transduction in adherent cells could be detected [15]. While, in the case of stimulation through an IgE antibodies, the negative results on non-atopic monocytes could be explained by the lack of IgE binding, it came as a surprise in the case of MoAb 15.1 F(ab)₂-biotin, which in all cases showed clearcut positivity. However, after neuraminidase treatment, not only was IgE binding restored but also a calcium

Table 3. Influence of culture conditions on the balance of receptor expression and IgE binding. ICAM-1 induction as an indicative for activation. Elutriated monocytes from a normal donor were stained after isolation (D0) and cultured overnight under the indicated conditions. CH (cycloheximide) was added at 1 µg/mL, IL-4 at 50 U/mL, IgE 2 µg/mL, MoAb 15.1 F(ab)₂-biotin and 15.1 F(ab)-biotin at 10 µg/mL. The results are given as percentage of positivity (%) and as MFI

Condition	D0 fresh		IMDM		IMDM CH		IMDM IL-4		IMDM IL-4 + CH		IMDM IgE		IMDM IgE + CH		IMDM 15.1 F(ab) ₂		IMDM 15.1 F(ab)	
	%	MFI	%	MFI	%	MFI	%	MFI	%	MFI	%	MFI	%	MFI	%	MFI	%	MFI
CD14	93	77	88	36	76	17	89	27	86	18	89	51	64	12	92	44	92	48
CD23	0	0	1	0	0	0	46	5	0	0	1	0	0	0	0	0	0	0
IgE/GaM	0	0	1	1	3	2	18	5	0	0	*13	*4	*18	*5	NT		NT	
ICAM-1	100	646	100	865	100	372	100	871	100	345	100	1803	100	667	100	1705	100	1707
15.1 F(ab) ₂ -b	20	4	1	0	NT		NT		NT		NT		NT		*27	*4.3	NT	
15.1 F(ab)-b	17	4	0	0	NT		NT		NT		NT		NT		NT		*11	*2

NT, not tested. * Adding second step MoAb only.

mobilization was observed in three out of four donors (Figs 5e–h).

Discussion

In the present study we demonstrated, that FcεRI is expressed on the cell surface of monocytes from all non-atopic individuals tested ($n=58$) at levels of up to 66% with a median of 18% (Fig. 1c). These results differed from previously published data [15] and prompted us to investigate this issue more closely. The expression of FcεRI, detected with the MoAb 15.1 (Fig. 1a), was confirmed with three other MoAbs, also targeting the FcεRI-α chain [5,11] and by Western blot of CD14 positive cells after MACS separation (Fig. 1b). We describe a way to stabilise and upregulate receptor expression, to influence the functionality of FcεRI and we present evidence that FcεRI is subjected to a constant turnover process which is slowed down but not prevented by the interaction with the ligand.

The unoccupied FcεRI on monocytes was rapidly down-regulated in culture media at temperatures above 4°C

(Figs 2a and b). Extensive isolation procedures in medium and raising the temperature above 4°C therefore run the risk of losing receptor expression, and indeed some donors only exhibited low initial levels of FcεRI (Figs 1c and 2b).

Surprisingly, FcεRI was not downregulated in PBS; on the contrary, there was a significant increase of expression (Figs 2c and d). Upregulation was detected after 1 h of culture, an effect that lasted more than 15 h, and viability and cell recovery were comparable after overnight culture in PBS or IMDM. The isolation procedures for PBMCs were minimized by applying ficoll separation only, subsequent washings being in cold PBS. The elutriation and further washings were also not carried out in culture media but in PBS. Maintaining the cells in PBS represents the major difference between our studies and previously published data [15] and while this tool is certainly distant from the *in vivo* situation, it is the first published means of stabilising FcεRI on antigen presenting cells.

To exclude the possibility that the FcεRI levels found were induced by conducting the isolation procedures in

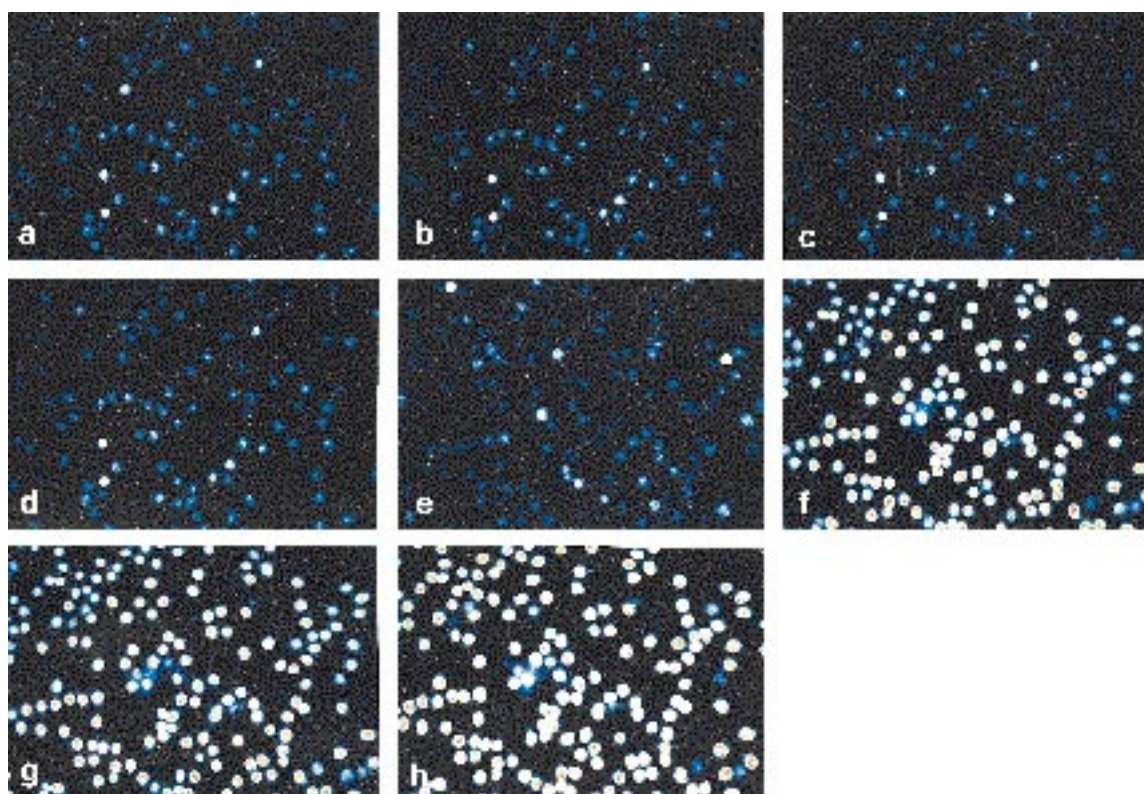


Fig. 5. Fluo-3 visualization representing calcium mobilization after FcεRI crosslinking on untreated or neuraminidase treated monocytes from a single non-atopic donor. Panels a–d. Untreated cells were activated with 16 µg/mL 15.1 F(ab)₂-biotin added at time 8 s and crosslinked with 10 µg/mL avidin added at time 64 s. The panels a–d represent 88 s, 112 s, 160 s and 184 s after activation, respectively. Panels e–h. Neuraminidase treated cells were activated as described above. The panels e–h represent 88 s, 112 s, 160 s and 184 s after activation, respectively.

PBS, although the cells were kept below 10°C, we incubated whole blood with MoAb 15.1 F(ab)₂-biotin, then applied ficoll-separation and compared the results to FcεRI expression stained after the normal isolation procedure within the same experiment. FcεRI expression was comparable. In addition, the FcεRI expression on CD14 positive monocytes after culture of whole blood for 15 h in the presence of IMDM or PBS followed the same pattern as demonstrated on isolated cells (data not shown).

We subsequently tested the influence of IL-4 on non-atopic monocytes. CD23 was induced both in IMDM and PBS, while FcεRI expression was observed only in PBS cultures (Table 1). This demonstrates firstly, that both culture conditions allow the monocytes to respond to extracellular stimuli, and secondly, that IL-4 is not involved in the regulation of FcεRI expression on these monocytes.

Essentially the same results were reproduced with monocytes from atopic donors, the only difference being a high level of *in vivo* bound IgE after isolation, in addition to unoccupied receptors (Table 2). As discussed for the monocytes from normal donors, monocytes from atopic donors displayed increased levels of unoccupied FcεRI in PBS and expression was lost in IMDM, showing that FcεRI is controlled by the same mechanisms on these monocytes. Interestingly, the level of *in vivo* bound IgE after overnight culture in IMDM was much lower compared with PBS, indicating that binding of IgE to FcεRI slows down the downregulation of the receptor, but does not prevent it (Table 2).

The influence of an 'atopic' environment on monocytes from non-atopic donors was assessed by adding IgE (2 µg/mL) to the overnight cultures. Cell-bound IgE was detectable under IMDM/IgE (Fig. 4a) and PBS/IgE conditions, but in PBS additional unoccupied receptor was found (data not shown). Culture in the presence of complete (Fig. 4b) or fragmented (Table 3) MoAb 15.1 also led to accumulation. CD23 was not induced under any culture condition (Table 2) and anti-CD23 MoAb did not have an accumulative effect (data not shown). The accumulation was higher with MoAb 15.1 than with IgE (Figs 4a and b). A possible explanation being that 15.1 binding slows down the disappearance of 'non-IgE-binding' FcεRI present at the time of isolation, demonstrated above for *in vivo* bound IgE on monocytes from atopic donors. As the addition of cycloheximide (1 µg/mL) did not abolish the IgE induced accumulation (Fig. 4a and Table 3), we conclude that the upregulation of FcεRI detected in this experimental setup is not due to *de novo* synthesis, but might be the consequence of a constant turnover process. The fact that monomeric IgE and MoAb 15.1 F(ab) fragments but not IL-4 induced an increase in the intensity of ICAM-1 expression suggests, that the mere binding of ligands to FcεRI causes a signal without the necessity for crosslinking (Table 3).

In spite of surface FcεRI expression on the monocytes from non-atopic donors, human IgE failed to bind significantly to the receptor (Fig. 3a). The same effect was observed using different sources of mouse IgE; biotinylated human Fcε fragments, however, clearly bound to untreated monocytes via FcεRI comparable to MoAb 15.1 (data not shown). It appears therefore, that the binding site for IgE on the α-chain of FcεRI is intact, but that secondary molecules prevent the binding of complete IgE molecules. Hence, we were left with the intriguing observation, that a major difference between monocytes from non-atopic and atopic donors lies in the binding of IgE to FcεRI, rather than in the expression of the receptor.

We therefore investigated the possibility, that the binding site for IgE was controlled or blocked by an unknown structure. Bieber *et al.* described, that the galectin-3 surface molecule had the capacity to modulate IgE binding to FcεRI on human Langerhans' cells [7]. However, in our study, lactose treatment to elute this structure did not affect IgE binding. Furthermore, galectin-3 was not detected on fresh monocytes with a specific antibody, suggesting that an involvement of galectin-3 in the regulation of FcεRI on normal monocytes can be excluded. Since sialyl-groups can exert steric hindrance to IgE binding [32,34], cells were treated with neuraminidase. This procedure restored the ability of FcεRI to bind IgE at significant levels (Fig. 3B). Competitive inhibition of positivity with either IgE or the neutralizing MoAb 15.1 verified FcεRI as the binding structure (Figs 3c and d). The enzyme treatment not only permitted the binding of IgE, but also enabled signalling via FcεRI, measured as calcium mobilization (Figs 5e-h). On untreated monocytes from healthy individuals crosslinking of FcεRI seems to be hindered, as MoAb 15.1-biotin did not lead to an activation of this signal transduction cascade in spite of the clearly detectable binding (Figs 5a-d). This was not a consequence of keeping the cells in PBS because at the same time calcium release was observed after cross-linking of FcγR [22]. Nevertheless, as emphasized for the culture in PBS, the effect of neuraminidase most likely does not directly reflect the *in vivo* situation but it illustrates that FcεRI on normal human monocytes can be functional under certain circumstances and therefore does not have an intrinsic defect.

Maurer *et al.* [15] proposed that the intensity of receptor expression within the monocyte population was related to allergy and that monocytes from atopic donors (after acid stripping to remove IgE from the cell surface) displayed high levels of FcεRI, whereas on non-atopic donors no or only moderate expression was observed. Taken together, our data demonstrate that individual differences of FcεRI expression can be seen on the monocytes and that receptor expression and function can be modulated. Serum IgE levels seem to play a critical role in the regulation of this

mechanism. We propose, that there is essentially no difference between monocytes from non-atopic and atopic donors and that the findings of Maurer *et al.* [15] can be explained by the following: firstly, the stabilizing effect of *in vivo* bound IgE on FcεRI. In the above mentioned study [15] the IgE bound to monocytes from atopic donors *in vivo* was only removed after the isolation procedure (by acid stripping) and not before. This effect is indirectly illustrated in Fig. 2a and Table 2, where in the first case, no FcεRI can be detected on normal monocytes after 15 h culture in IMDM, but monocytes from atopic donors still display positivity for *in vivo* FcεRI-bound IgE under the same conditions. In addition, we have shown that raising the temperature above 4°C during extensive isolation procedures in culture media reduces detectable FcεRI levels even further. Secondly, and most importantly, the monocytes from atopic donors will have accumulated FcεRI *in vivo* on the cell surface due to serum IgE levels (Table 2) and will therefore display elevated levels of FcεRI after acid stripping. Similar levels of expression can be obtained by overnight incubation of monocytes from healthy individuals in the presence of IgE.

To summarize, we propose that FcεRI is present on normal monocytes, that expression is rapidly downregulated under conventional culture conditions and that the receptor function is regulated by a structure sensitive to neuraminidase treatment. Further, we hypothesize that newly recruited FcεRI receptors reach the cell surface in a functional state which is lost when the ligand IgE, is not immediately present. This mechanism occurs in monocytes from both atopic and non-atopic donors. The differences in FcεRI levels observed between atopic and healthy individuals reported before [15], do not seem to be due to intrinsic differences concerning FcεRI expression or function, but to the serum IgE levels of atopics, that lead to the accumulation of IgE on the monocyte surface and consequently to cell–cell interactions. Future studies in our laboratory will address the nature and physiological relevance of the neuraminidase sensitive regulatory mechanism and possible receptor recycling, as this might lead to new insights into the causes of allergy and open new perspectives for drug development. However, our results clearly indicate that the mere presence of FcεRI on monocytes is not related to allergy or atopy.

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