

Regulation of FcεRI expression on human monocytic cells by ligand and IL-4

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Abstract

Background The FcεRI subunit composition and kinetic of expression differ between antigen-presenting cells and mast cells. Up to now, there has been no human *in vitro* model available that mimics the characteristics on monocytes.

Objective The characterization of a natural human monocytic cell line (THP1), which expresses FcεRI, and the comparison to primary human monocytes and other monocytic cell lines, which only express FcεRI after transfection with the human FcεRI α-chain gene.

Methods Surface receptor expression was characterized by flow cytometry, the human FcεRI α-chain gene was introduced by electroporation, and induction of FcεRI α-chain message was detected by semiquantitative RT PCR.

Results Here we show that the parental human cell line THP1, but none of the other cell lines tested, displays surface FcεRI in response to IL-4 or incubation with receptor ligand (IgE, antibody). Transfection of FcεRI α-chain resulted in receptor expression on all cell lines, all of which increased surface FcεRI in the presence of IgE. Only the THP1-α transfectant, however, further increased receptor levels in response to IL-4, resulting from mRNA induction for the FcεRI-α, but not the β- or γ-subunit.

Conclusion Based on THP1, U937 and HL60 and their α-chain transfectants we present a model system for the study of FcεRI regulation and signalling on human cells. THP1 in particular, due to its responsiveness to both ligand and IL-4, even without prior manipulation, is ideally suited to address questions on FcεRI modulation in an 'allergic environment'.

Keywords: cell line, FcεRI, human, IgE, IL-4, monocytes, regulation, transfection

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Introduction

The high affinity receptor for IgE, FcεRI, is one of the key structures involved in allergic inflammation (reviewed in [1]) and has therefore become a focus of immunology research. Human FcεRI expression, initially thought to be limited to mast cells and basophils, is progressively detected on more human cell populations, amongst them Langerhans' cells [2,3], dendritic cells [4] and monocytes [5,6], platelets [7] and bronchial epithelial cells [8]. Whereas the effector cells carry the αβγγ-FcεRI complex [5,9], the

presence of β-chain protein in dendritic cells and monocytes is still debated [4,10], and could influence FcεRI function [11]. This concerns in particular the amplitude of the induced signal [12] and the stability of the receptor complex on the membrane [5].

The 'classical' model-system for FcεRI-related studies are rat basophilic leukaemia (RBL) cells, mainly due to the stable and relatively high-level receptor expression on these cells, but also due to the lack of human cell lines expressing FcεRI. The recent establishment of human monocytic cell lines with stable FcεRI expression has opened new possibilities [12,13]. These will include in particular the investigation of regulatory mechanisms controlling the expression of the αγγ in comparison to the αβγγ complex (current

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unpublished results indicate these differences). In addition, it still remains to be shown that the same signalling molecules activated by FcεRI on RBL cells are also present in human cells and linked to the receptor (reviewed in [14]), a situation obviously mimicked more closely in a human cell line.

In the present study, we compare the regulation of FcεRI expression and the response to stimuli related to allergy between: (1) human peripheral blood monocytes from normal donors; (2) THP1 and the recently published THP1-α cells and; (3) the cell lines HL60 and U937 and (newly established) FcεRI-expressing transfectants of these lines (HL60-α and U937-α lines).

Material and methods

Reagents and antibodies

IgG1-FITC isotype-control and streptavidin-PE, Becton Dickinson (CA, USA); CD14-FITC and CD64, Serotec (Kidlington, Oxford, UK), CD23-FITC and CD32, Immunotech (France), goat antimouse PE (GaM-PE), Tago (CA, USA). Antihuman FcεRI α-chain monoclonal antibodies (mAbs): 15.1 hybridoma (isotype IgG1) was kindly given by Dr J.-P. Kinet (Beth Israel Hospital, Boston, MA, USA) [2], F(ab)2 and Fab fragments were generated using standard techniques. 6F7 and 22E7 (both IgG1) were gifts from Dr R. Chizzonite (Hoffmann-La Roche, NJ, USA) [15]. Hapten-specific NIP-chimaeric IgE was purchased from Serotec (GB), 96-well plates from Costar (Cambridge, MA, USA), Micronic(r) tubes from Micronic systems (NL), 7-aminoactinomycin D (7-AAD), Molecular Probes (OR, USA) and hygromycin B, Calbiochem (CA, USA).

Human recombinant interleukin-4 (hrIL-4, 1 U = 80 pg) and GM-CSF (6.13 × 10⁶ U/mg) were obtained from Sandoz Pharma AG (CH), hrIL-13 from R & D Systems (Abingdon, UK), hrIFN-γ (1 U = 100 pg) from Genzyme (MA, USA) and hrIL-9 from PreproTech Inc. (NJ, USA).

Cell culture, DNA and transfection

The cell lines were routinely cultured in RPMI 1640 (Gibco BRL, MD, USA) supplemented with 100 μM L-glutamine, 5000 U/mL penicillin, 5000 μg/mL streptomycin and 10% fetal calf serum (FCS) in a humidified, 5% CO₂ incubator at 37 °C, the Burkitt-Lymphoma cell line BL2 [16] was kept in Iscove's Modified Dulbecco's Medium (IMDM) containing 10% FCS. The generation of the FcεRI-α expression vector was described before, as was the transfection by electroporation [13].

Flow Cytometric (FACS) analysis of cell surface receptors

Cell labelling was performed as previously described [5].

Measurements included isotype controls and a viability measurement with 7-aminoactinomycin D and were analysed on a FACScan (Becton Dickinson, CA, USA) equipped with 'Lysis II' version 1.0 software. Percent positivity is given as the percentage above the isotype control, the fluorescence intensity is indicated as mean fluorescence index (MFI), representing the fluorescence intensity of the ungated sample minus the intensity of the relevant control.

Semiquantitative reverse transcription polymerase chain reaction (RT PCR) for the detection of FcεRI α-, β- and γ-chain transcripts

Total RNA was prepared with TRIzol reagent (Gibco BRL, MO, USA) according to the manufacturer's protocol. cDNA preparation was performed using 15 μg of RQ1 DNase (Promega) treated RNA. Reverse transcription (RT) was initiated with 0.4 μg of oligo(dT) (Amersham Pharmacia Biotech AB, Uppsala, Sweden), 0.4 μg of random primer (Gibco BRL) and 25 U avian myoblastosis virus reverse transcriptase (Pharmacia) in a total volume of 50 μL at 42 °C for 50 min cDNA was denatured at 95 °C for 5 min. PCR was performed in a final volume of 100 μL including 1 μL of cDNA per reaction mixture in a Techne PHC3 thermal cycler. The PCR reaction mixture was denatured for 40 s at 95 °C, annealed at 60 °C for 40 s and extended at 72 °C for 1 min. The conditions of the PCR reaction were optimized to ensure a semiquantitative readout.

The quality and quantity of the cDNA was tested with glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-specific primers, while KU812 cDNA (human basophilic cell line) served as positive control at the maximum amount of cycles and PCRs without cDNA as negative controls.

PCR products were fractionated on 1% agarose gels, transferred onto Hybond-N nylon membranes (Amersham) according to Sambrook *et al.* [17] and fixed by UV irradiation. Southern hybridization was carried out according to Chujor *et al.* [18] with specific oligonucleotides labelled at their 3' ends with a terminal transferase kit (Roche Austria GmbH, Vienna, Austria). Southern blots were exposed to KODAK BIOMAX MR films up to 24 h.

Statistics

Significance of differences between culture conditions was calculated using paired *t*-tests and focusing on the one-tailed *P*-values (ns = not significant, **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001). Calculations were performed for percentage positivity and MFI, and accepted as truly significant only if the *P*-values for percentage and MFI were ≤ 0.05, to

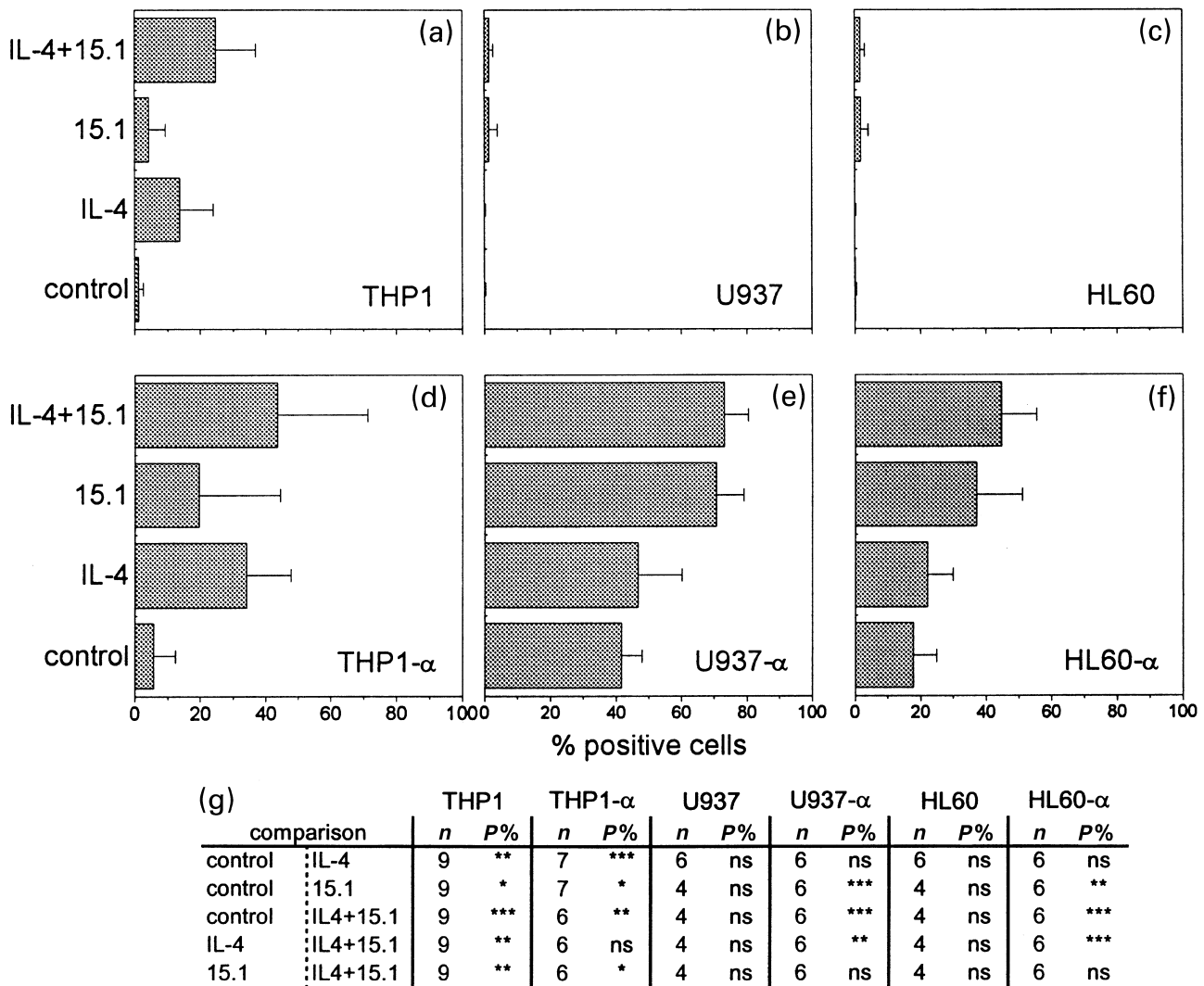


Fig. 1. Summary of experiments conducted on the various cell lines. In this summary figure, the bars depict the percentage FcεRI positivity above isotype control and the error bars show the standard deviation, while the number of repeats is given in panel G. IL-4 was used at 25 U/mL, mAb 15.1-b F(ab)2 at 2.5 µg/mL on THP1 (graph a), U937 (b), HL60 (c), THP1-α lines of variable expression (d), U937-α (e) and HL60-α (f). For clarity of display, the one-tailed significances calculated in paired *t*-tests for percentage positivity between the various conditions of culture and the number of experiments included in the calculation are presented separately in a panel G (ns = non significant, **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001).

exclude artefacts caused by the geometry of the FACS-peaks.

Results

Ligand-induced FcεRI surface expression

Previous observations on primary monocytes [5], led us to hypothesize a cycling mechanism for FcεRI on antigen-presenting cells [1], in particular the rapid loss of expression upon cell isolation and cycloheximide-insensitive receptor-accumulation in the presence of ligand [5].

FcεRI expression after culture in the presence or absence of FcεRI ligands (IgE or fragments of the monoclonal antibodies (mAb 15.1) was studied on the human monocytic cell lines THP1, U937 and HL60, as well as the THP1-α lines, established previously through stable transfection of THP1 with a FcεRI-α cDNA plasmid [13]. In addition, following the previously described protocol [13], several U937-α and HL60-α cell lines with stable FcεRI expression were established and included.

Whereas U937 and HL60 remained negative for FcεRI after overnight culture with mAb 15.1 F(ab)2 (Fig. 1b,c), a significant positive peak-shift was detected on THP1 (Fig.

1a,g) and an even more pronounced increase of FcεRI levels was observed on the THP1-α, U937-α and HL60-α transfectants (Fig. 1d,e,f,g). No effect on FcεRI expression was seen in the mouse IgG1 (15.1 isotype) control cultures at the identical concentration (data not shown)

Cytokine-induced FcεRI expression

The effect of various interleukins on FcεRI expression was tested in subsequent experiments, but only IL-4 (Fig. 2a) or IL-13 (data not shown) induced a dose-dependent, significant induction of FcεRI on THP1 and THP1-α, but on no other cell line tested (Fig. 1). The IL-4-induced (upregulated) FcεRI on THP1 and THP1-α was able to mediate antigen presentation through IgE in a system as described by Maurer *et al.* [4] (data not shown; Dubois *et al.*, manuscript in preparation). Maximal THP1-FcεRI expression after IL-4 culture was reached at a concentration of ≥ 10 U/mL (Fig. 2a) and to ensure optimal levels, IL-4 was used at 25 U/mL in further experiments. Similarly, CD23 induction peaked at this IL-4 dosage on THP1 cells (Fig. 2b) and all cell lines tested (data not shown). Surface FcεRI expression appeared as early as 4 h after IL-4 addition, with a maximum between 10 and 20 h, and levelled off thereafter, when, interestingly, CD23 levels still increased (Fig. 2b). Subsequently FcεRI receptor expression diminished in spite of optimal IL-4 concentrations or upon removal of IL-4. The combination of IL-4 and IL-13 at optimal concentrations did not lead to a further increase. Similar percentages of FcεRI expression were detected with the FcεRI-α-specific antibodies 6F7, 22E7 [15] and 15.1 F(ab)2, F(ab) [2] (data not shown). The FcεRI γ-chain is not a limiting factor in this system, indicated by comparable levels of both CD64 and CD32 on all cell lines and unchanged FcγR expression before and after transfection [13] or stimulation with IL-4 (data not shown). The observed low CD14-levels on THP1 were not altered by the IL-4 treatment, arguing against an involvement of cell differentiation particularly since FcεRI expression was similar on CD14 positive or negative cells (data not shown). Soluble or cleaved FcεRI-α was not found in the cell culture supernatant.

IFN γ on its own had no effect on FcεRI expression, but dose-dependently counteracted IL-4 on both THP1 (Fig. 2c) and THP1-α (data not shown), regardless of whether IFN γ was simultaneously present or washed away after pre-incubation (Fig. 2c).

FcεRI-α chain mRNA is increased by IL-4 on THP1

A semiquantitative RT PCR protocol was developed to address the subunit involvement in IL-4-induced FcεRI expression. The PCR primer pairs, hybridization primers and number of cycles used in the reaction are summarized in

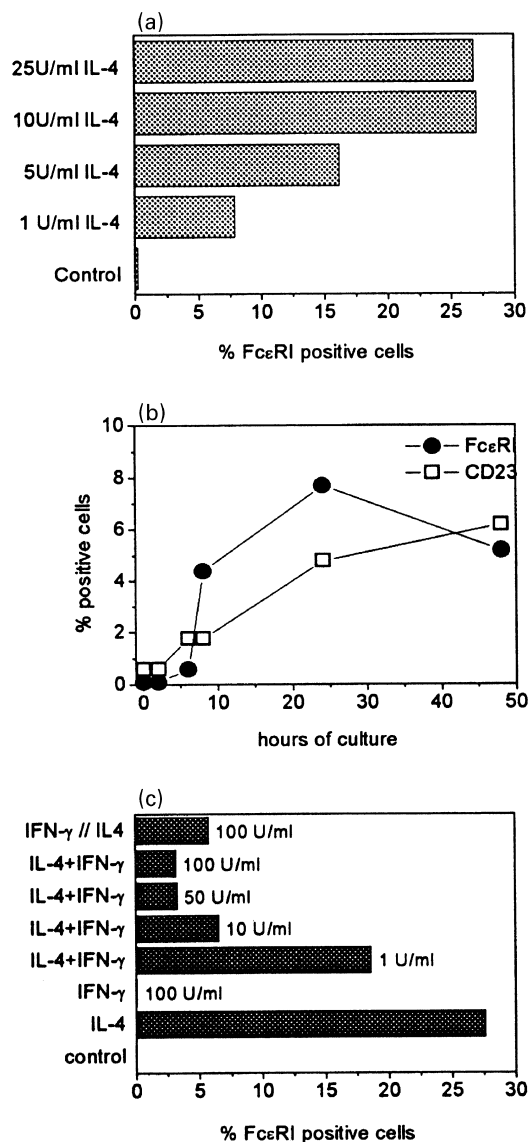


Fig. 2. IL-4-induced FcεRI surface expression and effect of interferon-γ. The figure illustrates on the parental THP1 cell line, the dose-dependent induction of surface FcεRI by IL-4 in a representative FACS experiment (panel a), a time curve of FcεRI and CD23 induction (panel b) and the effect of interferon-γ (panel c). The indicated concentration of IFN γ (1U = 100 pg) was added to overnight cultures with IL-4 (25 U/mL, 1U = 80 pg). The top bar illustrates FcεRI induction after 1-day incubation with IFN γ (100 U/mL), followed by washes, and subsequent overnight culture with IL-4. Shown is the percentage of positive cells above the isotype control in FACS analysis.

Fig. 1a. The signals for the FcεRI α-, β- and γ-chains were compared with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) signal, as a quality and quantity control of the cDNA preparations. Only FcεRI-α transcripts were induced upon IL-4 treatment, neither the β- nor the γ-subunit signals were modulated (Fig. 1b). In agreement

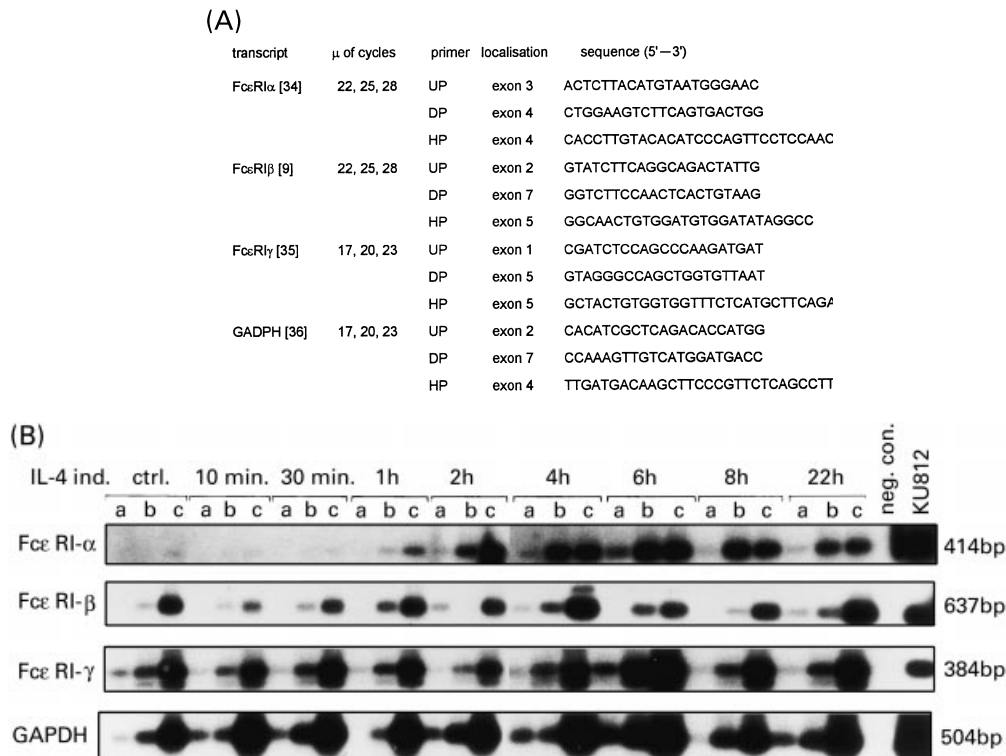


Fig. 3. Semiquantitative RT PCR for the detection of THP1 FcεRI α -, β -, and γ -chains after induction with IL-4. RT PCR and Southern hybridization were performed as described in materials and methods, the sizes of fully spliced PCR products are indicated on the right. Number of amplification cycles (a, b, c), PCR primer pairs (UP = upstream, DP = downstream) and hybridization primers (HP) used are summarized in panel a. Cells were stimulated with 25 U/mL IL-4 and the expression of FcεRI- α -, β -, and γ -mRNA was determined at various timepoints (b).

with the FACS FcεRI surface staining, the maximum FcεRI α -chain signal was observed at 4 h and slowly decreased after overnight stimulation, which parallels the reported kinetic on eosinophils [19]. Low levels of FcεRI- β transcripts were consistently detected in THP1 (Fig. 1b) and all other monocytic cell lines tested (data not shown). Clearly, however, the FcεRI expression, detected on the surface of THP1 in response to IL-4, is preceded and mediated by increased α -chain mRNA only.

Accumulation and induction

The THP1- α , U937- α and HL60- α lines, together with their parental lines, were cultured with ligand, IL-4 and the combination of both. The results are described in Figures 1 and 4 and show that, of the parental lines, THP1, but not U937 or HL60, respond to either ligand or IL-4 with significant FcεRI surface expression, but especially to the combined condition. Amongst the transfectants THP1- α retains the responsiveness to IL-4, while all transfectants display a significant increase of FcεRI in response to ligand. In addition U937- α displays a minimal, but significant FcεRI increase in IL-4 + 15.1 vs 15.1 alone (Fig. 1e,g).

The statistical significance of differences between the various culture conditions and the number of repeats are summarized in Figure 1g.

To determine the involvement of *de novo* synthesis, 2 μ g/mL cycloheximide were added to the control and various culture conditions of THP1, U937- α and HL60- α . IL-4- and ligand-induced FcεRI expression on THP1 were greatly reduced by cycloheximide and less so in the combined condition (Fig. 4a), although that was the case for CD23, which served as an internal control on all cell lines (data not shown). This indicates that the increase in surface FcεRI is not exclusively mediated by *de novo* synthesis, an observation supported by similar findings on U937- α and HL60- α : the increased FcεRI expression occurring in the presence of 15.1 F(ab)2 or F(ab), was reduced to, but not below the medium- and isotype control (IgG1) baseline in the presence of cycloheximide (Fig. 4b).

Discussion

The classical FcεRI *in vitro* model is based on rat basophilic leukaemia (RBL) cells and has provided valuable results in this line of research. However, alternative tools with

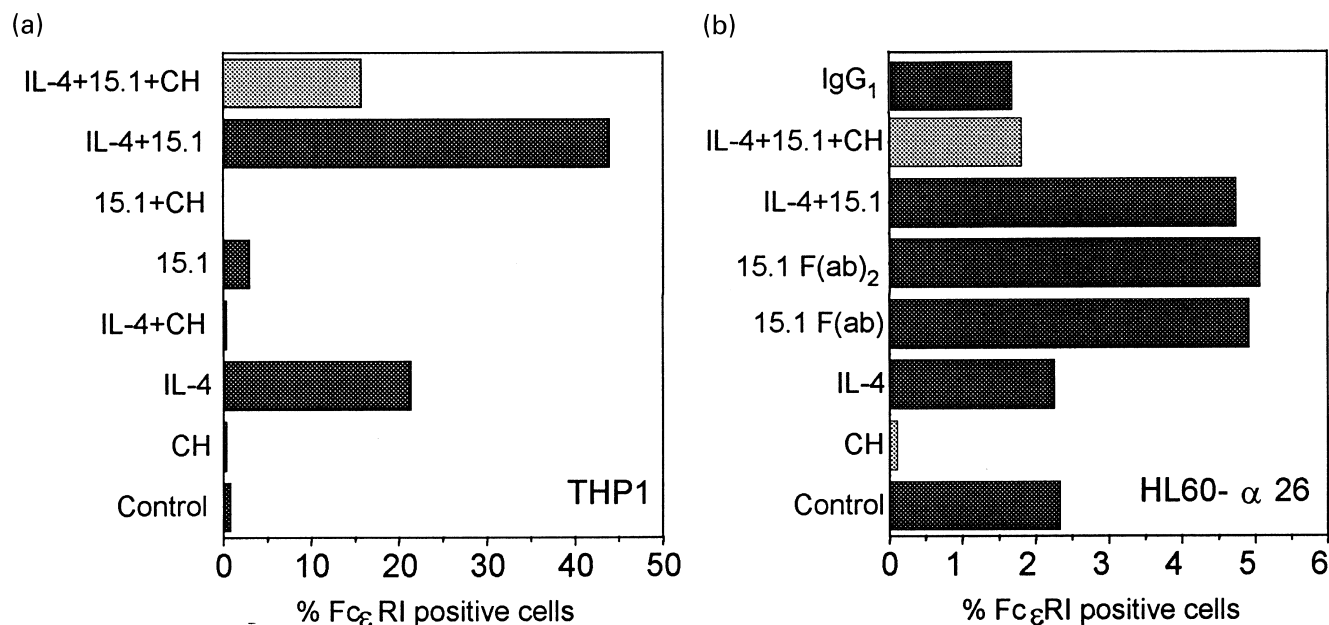


Fig. 4. Influence of cycloheximide on Fc ϵ RI surface expression. The influence of cycloheximide (CH, 2 μ g/mL) on Fc ϵ RI modulation is shown for various culture conditions on THP1 (a) and HL60- α 26 (b) in representative experiments. Cells were cultured in the presence or absence of IL-4 (25 U/mL), 15.1-b F(ab)₂ (standard protocol) or F(ab) (both at 2.5 μ g/mL), mouse IgG₁ (2.5 μ g/mL) and CH (grey bars).

perhaps more relevance for the human system have now become available with the establishment of human Fc ϵ RI expressing cell lines.

In the present study, Fc ϵ RI expression was investigated on the human monocytic cell lines THP1, HL60 and U937 and on Fc ϵ RI expressing stable α -chain transfectants of each of these (THP1- α , HL60- α and U937- α lines), generated as reported earlier for THP1- α [13]. HL60 and U937 were included for the following reasons: (i) the Fc ϵ RI- α chain cDNA used for the construction of the plasmid [13] was initially isolated from HL60 (ii) HL60 reportedly expresses Fc ϵ RI- α , - β and - γ transcripts upon differentiation with butyrate [20], and (iii) U937 was used in a similar set-up by others [12], cotransfecting the γ -chain with Fc ϵ RI α - or α - and β -chains. Since the $\alpha\gamma\gamma$ complex forms the minimal surface Fc ϵ RI requirement on human cells [21], we conclude that in all three cell lines the transfected α chain assembled with the endogenous γ -chain [22–24], apparently present in sufficient amounts.

We demonstrate ligand-induced upregulation of Fc ϵ RI as a general mechanism of those human monocytic cells capable of Fc ϵ RI expression. It was observed in the presence of IgE or a monoclonal antibody against the Fc ϵ RI α -chain independently of whether the receptor was expressed naturally (primary monocytes, THP1) or introduced by transfection of Fc ϵ RI- α chain cDNA (THP1- α , HL60- α and U937- α lines). In addition, the accumulative effect was observed with mAb 15.1 F(ab)₂ or F(ab) fragments, which indicates

that cross-linking of receptors is not required (Fig. 4). The effect of ligand differs in its sensitivity to cycloheximide, since the inhibitor has no effect on monocytes [5], whereas accumulation is greatly reduced on the cell lines (Fig. 4), indicating that both receptor turnover and de novo synthesis contribute to their observed surface expression. Similar results have been published for human basophils [25–27] and mast cells [28,29].

On THP1 and THP1 α , but on no other cell line tested, IL-4 (Fig. 2a) and IL-13 led to the dose- and time-dependent (Fig. 2b) induction of Fc ϵ RI, in spite of all parental cell lines carrying Fc ϵ RI- α message [13]. The kinetic of IL-4-induced Fc ϵ RI expression (Fig. 2b) indicated the mobility of the receptor, while IFN γ antagonized the action of IL-4 (Fig. 2c). Further insight into the action of IL-4 was provided by semiquantitative RT-PCR, demonstrating that only α -chain message increased in time, while no effect on either the Fc ϵ RI β -chain or γ -chain transcripts was visible. Importantly, the down regulation of Fc ϵ RI expression by IFN γ was also seen at the mRNA level (data not shown). This is in agreement with a recent report which demonstrates that the inhibitory action of IFN γ on IL-4 signals in general and STAT6 in particular, is due to the induction of SOCS-1 gene expression by IFN γ [30]. SOCS-1 has previously been characterized as an inhibitor of IL-4 signal transduction [31] and belongs to the family of Suppressors Of Cytokine Signalling and acts through the binding to the JAK family of protein tyrosine kinases and inhibiting their intrinsic

enzymatic activity (reviewed in [32]). The α -chain message peaked at 4 h, followed by a slight decrease (Fig. 3), similar to observations on human eosinophils [19], and in parallel to the kinetic of surface FcεRI (Fig. 2b), with the expected delay due to protein synthesis and receptor assembly. The presence of FcεRI β -chain transcripts in THP1 is in contrast to earlier published results [4,6], and likely due to increased sensitivity of detection in the present study. We currently have no results on FcεRI β -chain protein in the cell lines and the possibility exists that at least a small part of the detected FcεRI is expressed as $\alpha\beta\gamma\gamma$ complex. However, we hypothesize the $\alpha\gamma\gamma$ complex to be predominant, especially after IL-4 induction. At the moment we cannot distinguish between IL-4-induced increase of FcεRI- α transcription vs stabilization of the mRNA, but the magnitude and the time-kinetic of the observed response suggest the cause to be increased transcription initiation.

The combined stimulus of ligand and IL-4 has an additive effect on THP1 and THP1- α , arguing for an involvement of separate signalling pathways (Figs 1a, 1d and 4a). The findings on THP1 thus mimic results on Langerhans cells [11], eosinophils [19] and mast cells [33], whereas the results on U937- α and HL60- α lines are comparable to previous findings on primary monocytes [5].

In conclusion, we present THP1 as the first human (monocytic) cell line with natural FcεRI expression at levels comparable to primary monocytes. THP1 and the THP1- α lines share the capability of ligand-induced accumulation and IL-4 induced FcεRI expression (Figs 1 and 4) indicating a special position for THP1 amongst the myelomonocytic cell lines. A further advantage of THP1, in regards to FcεRI-related studies, are the low CD23 levels (Fig. 2b) on unstimulated cells.

The availability of related cell lines that share certain features with THP1, such as the ligand-induced increase of FcεRI on HL60- α and U937- α permits the comparative and detailed investigation of FcεRI turnover and regulation. Thus further advances in the knowledge on FcεRI and the identification of structural elements involved in the regulatory processes will be facilitated.

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