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Fc alpha-receptor expression on the myelomonocytic cell line THP-1: comparison with human alveolar macrophages

Y. Sibille*, S. Depelchin*, P. Staquet*, B. Chatelain**, P. Coulie†, L. Shen††, C. Vander Maelen*, J.P. Vaerman*

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ABSTRACT: Immunoglobulin A (IgA) and alveolar macrophages are two important components of the immune system in the respiratory tract. Fc alpha-receptors (FcαR) are present on neutrophils, eosinophils and a series of human mononuclear phagocytes, including monocytes, alveolar macrophages and leukaemia cell lines (U-937).

In the present study, using idiotypes and anti-idiotypic antibodies, we report that THP-1 cells, a myelomonocytic cell line, constitutively express FcαR and that all IgA preparations used bind the receptor. Of the stimuli used (phorbol myristate acetate, retinoic acid, calcitriol), only calcitriol can induce differentiation of THP-1 cells, as assessed by CD14 expression. The expression of FcαR appears to be independent of cell differentiation, since calcitriol pretreatment has no effect on IgA-binding. Finally, My43, a monoclonal antibody recognizing the FcαR on U-937 cells, does not bind to THP-1 cells or to human alveolar macrophages. In addition, preincubation of THP-1 cells or human alveolar macrophages with My43 does not diminish IgA-binding to these cells. Ribonucleic acid (RNA) encoding the FcαR isolated from U-937 is expressed, although possibly at a lower level, in alveolar macrophages and THP-1 cells.

In conclusion, FcαR are constitutively expressed on THP-1 cells and share some characteristics with the FcαR described in human alveolar macrophages. THP-1 cells, therefore, may represent a reasonable model for further investigation of the interaction of immunoglobulin A and tissue macrophages.

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*Experimental Medicine Unit, **Laboratory of Haematology, †Ludwig Cancer Research Institute, International Institute of Cellular and Molecular Pathology (ICP), Catholic University of Louvain, Brussels, Belgium. ††Dept of Microbiology, Dartmouth Medical School, Hanover, New Hampshire, USA.

Correspondence: Y. Sibille
Pneumology Department
University Hospital of Mont-Godinne
B-5530 Yvoir
Belgium

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The defence mechanisms of the lower respiratory tract involve both cellular and humoral immune components. In particular, alveolar macrophages (AMs) represent the resident phagocytes of the bronchoalveolar space, and account for the majority of clearance of particles and micro-organisms reaching the distal airways [1]. As part of the humoral immunity, immunoglobulin A (IgA) is the predominant immunoglobulin isotype in mucosal secretions, including bronchial epithelial lining fluid. It has long been recognized that IgA neutralizes bacterial and viral antigens in external body fluids, preventing absorption of these noxious products. More recently, additional functions have been assigned to mucosal IgA, and, specifically, the removal of antigens from the lamina propria and from epithelial cells has been demonstrated to be mediated, at least in part, by the transepithelial transport of IgA with the polymeric immunoglobulin receptor (pIgR) [2, 3].

A receptor for the Fc fragment of IgA (FcαR), different from the pIgR, has been identified and characterized on several human phagocytic cells, including neutrophils, eosinophils, monocytes, leukaemia cell lines (HL-60 and U-937) and AMs [4–8]. Although the definite role of the FcαR remains uncertain, IgA has been shown to modulate numerous phagocyte functions, such as mobility, phagocytosis, oxygen radical release and tumour necrosis factor-α (TNF-α) production [9–12].

THP-1 is a well-established cell line derived from a child with acute monocytic leukaemia [13]. This cell line appears to be blocked at a relatively late stage of normal differentiation, and is committed to differentiate *in vitro* into the monocyte/macrophage lineage, in particular in the presence of the phorbol ester, phorbol-myristate acetate (PMA) [14, 15]. THP-1 cells, therefore, represent a useful model for investigation of mononuclear phagocyte properties. Earlier studies on THP-1 cells have already recorded that differentiated cells are capable of releasing oxygen radicals and cytokines, of exhibiting phagocytic and microbicidal activity, and of expressing surface receptors for a series of ligands, including the complement component C3b and immunoglobulin G (IgG) [13, 16]. Moreover, THP-1 has been used by others to better characterize the enzyme-releasing peptide, a human AM secretory product [17].

In order to continue the study of FcαR on human tissue macrophages, and considering both the difficulty to obtain large numbers of human AMs and their various phenotypes among donors, we evaluated whether THP-1 cells express the FcαR, and then compared the characteristics of FcαR on THP-1 cells with those already described for human AMs.

Material and methods

Cells

The promyelocytic cell lines THP-1 and U-937 were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were plated in 75 cm^2 Falcon plastic flasks, containing RPMI 1640 supplemented with 5% foetal bovine serum, 2 mM L-glutamine, 100 $\mu g \cdot ml^{-1}$ streptomycin, and 100 $U \cdot ml^{-1}$ penicillin (medium), at 37°C and 5% CO_2. The cells were subcultured every third day, at an initial density of 1×10^5 $cells \cdot ml^{-1}$.

THP-1 cells were further cultured in the presence or the absence of the following agents: PMA, retinoic acid (Sigma, St Louis, MO, USA) and 1,25-dihydroxyvitamin D_3 or calcitriol (Hoffman La Roche, Basel, Switzerland). Time and concentration curves were performed for each agent in the medium.

Human AMs were obtained from four normal nonsmoking volunteers by bronchoalveolar lavage (BAL), as described previously [18].

Immunoglobulin preparations

Human IgG and IgA immunoglobulins. Polyclonal (PC) IgG was isolated from normal pooled serum by diethylaminothanol (DEAE)-cellulose chromatography, at pH 8.0 in 0.04 M Tris-HCl buffer. PC serum dimeric-IgA1 (d-IgA1) was purified from the serum of a patient with a marked elevation of PC IgA [19], by a combination of gel-filtration on Ultrogel AcA22, preparative zonal electrophoresis on Pevikon [20], and affinity chromatography on Jacalin-Sepharose [21, 22]. PC milk secretory IgA (sIgA) was prepared as described previously [23]. Monomeric-IgA (m-IgA) and dimeric or polymeric (p-IgA) fractions of IgA1 and IgA2 monoclonal (MC) proteins (d-IgA1λ (Cr); m- and p-IgA1λ (Cl); m- and p-IgA2κ(Pa)) were isolated from myeloma sera, as described for PC serum IgA1, with occasional addition of a passage through one or several of the following immunosorbent columns (prepared with CNBr-activated Sepharose, Pharmacia, Uppsala, Sweden), made with monospecific antisera against α_2-macroglobulin, haptoglobin, albumin, α_1-antitrypsin, C3, C4, γ-chains and/or μ-chains [24, 25], to remove the corresponding contaminating proteins. The preparation of Fcα fragments of MC m-IgA1 (Cl) was the same as that used previously [9].

Purity of immunoglobulins (at 5–10 $mg \cdot ml^{-1}$) was checked by immunoelectrophoresis and/or Ouchterlony analysis, using a battery of monospecific antisera against serum proteins, as described elsewhere [24, 25]. Some samples were also run on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) after reduction [23], to confirm the presence of α-chains and light chains, and of secretory component for milk sIgA. Size distribution of MC and PC IgA preparations into m-, d- or larger p-IgA was assessed by sucrose density gradient ultracentrifugation, as described elsewhere [24, 25]. Protein quantitation in phosphate or borate-buffered saline, pH 7.2 or pH 8.4, was carried out by measuring optical density at 280 nm in a l.0 cm cuvette, using 13.4 or 13.8 as absorption coefficients of 10 $mg \cdot ml^{-1}$ solutions for IgA or IgG, respectively.

Control mouse MC IgM and My43 anti-FcαR IgM. Purified mouse IgM, a control MC IgM protein without known antibody activity, was purchased from Coulter, Luton, Bedfordshire, UK (Cat. No. 66024-02). Mouse MC IgM antibody My43 (Batch 51) against monocyte FcαR was prepared as described previously [12], and stored frozen as undiluted culture fluid in RPMI 1640.

Rabbit normal IgG and anti-IgA (isotype-specific) and anti-IgA (idiotype-specific) antibodies. Three different monoclonal IgA proteins (IgA1 Cl, IgA1 Cr, IgA2 Pa) were used to immunize a different rabbit in order to prepare three different samples of anti-idiotypic (Id) antibodies. After multiple intramuscular injections with complete, and later incomplete, Freund's adjuvant, the rabbits were exsanguinated when their sera were shown to give strong precipitation arcs upon immunoelectrophoreses with the immunizing MC IgA, as well as with PC serum IgA, and when, in addition, a strong spur was observed when the homologous MC Id-IgA was compared in Ouchterlony tests with several other MC IgA, as well as with PC serum and sIgA. These rabbit sera were used to prepare: 1) anti-Id antibodies (anti-IdA1 (Cl), anti-IdA1 (Cr) and anti-IdA2 (Pa)); 2) anti-IgA isotype-specific antibodies (anti-Iso-IgA); as well as 3) normal control rabbit IgG, devoid of anti-human-Ig antibody activity. Anti-Iso-IgA antibodies were first isolated by passage of the rabbit serum through a column of Sepharose beads covalently coupled to a mixture of various MC IgA proteins of both subclasses and light chain types; the retained antibodies, after elution with 0.1 M citrate buffer pH 2.3, neutralization and concentration by Amicon pressure ultrafiltration, comprised a mixture of antibodies against α-chains as well as against κ- and λ-chains. Anti-light chain antibodies, as well as possible contaminating anti-γ-chain antibodies, were removed by passage through a column of PC IgG-Sepharose, allowing recovery in the unretained fraction of purified anti-Iso-IgA antibodies. Anti-Id antibodies were purified from the unretained fraction of each rabbit serum after its passage through the mixed IgA-immunosorbent. The unretained serum was passed through an immunosorbent made with the corresponding purified Id-IgA, and the retained anti-Id antibodies were then eluted with acid buffer, neutralized and concentrated as above. Finally, rabbit serum proteins unretained on the Id-IgA immunosorbent were passed through a column of protein A-Sepharose, and the residual

normal rabbit IgG, a control devoid of anti-human Ig antibody activity, was also eluted at acid pH, neutralized and concentrated.

Rabbit control IgG, as well as anti-Iso-IgA and anti-Id antibodies, were extensively tested, at a concentration of ≥5.0 mg·ml^{-1}, for their reaction (or lack of reaction) by immunoelectrophoresis and Ouchterlony immunodiffusion tests against the various samples of MC and PC IgA mentioned above, as well as against normal human whole serum. In particular, anti-Id antibodies were shown to be unreactive against PC serum IgA and sIgA, but also against more than 10 other MC IgA proteins comprising m- and p-IgA of both subclasses and light chain types, all at concentrations from 0.1 to ≥5.0 mg·ml^{-1}. Occasionally, results of these tests indicated that further immunoabsorptions were needed to obtain the required specificity.

Rabbit F(ab')$_2$ fragments. These were prepared from control rabbit IgG as well as from the anti-Iso-IgA and anti-Id antibodies, by digestion of at least 20 mg of rabbit IgG with 0.4 mg of crystalline pepsin in 2 ml of 0.1 M Na acetate buffer, pH 4.5 for 16–20 h at 37°C; digestion was stopped by raising the pH to 8.0 with solid Tris base, and the digest was then gel-filtered on a column of Ultrogel AcA 44 to separate undigested IgG and IgG aggregates from F(ab')$_2$ and Fab' fragments. Purity of F(ab')$_2$ fragments was checked by their lack of precipitation by Ouchterlony immunodiffusion with goat antiserum specific against rabbit Fcγ.

Fluorescent conjugates. The different F(ab')$_2$ preparations (anti-Iso-IgA, anti-Id and normal control) were concentrated to 5–10 mg·ml^{-1} before conjugation with fluorescein isothiocyanate (FITC), as described previously [7]. The fluorescein/protein (F/P) ratio of the FITC-conjugates was determined according to the normogram of THE [26], and their protein contents were determined by absorption measurements at 280 and 495 nm, as described by BRANDTZAEG [27]. The F/P ratios were 2.5, 2.4, 2.3, 1.7 and 1.1, respectively, for control (non-anti-IgA), anti-Iso-IgA, anti-IdA1 (Cr), anti-IdA1 (Cl) and anti-IdA2 (Pa), F(ab')$_2$ frag-ments.

Rat IgM MC antibody (LO-MM-3) against mouse IgM was generously provided by H. Bazin (IMEX, UCL, B-1200 Brussels); after FITC-labelling, its F/P ratio was 0.75.

The FITC-conjugates of mouse MC anti-CD4 IgG1 (Cat. No. 7323) and anti-CD14 IgG2b (Cat. No. 7493) were purchased from Becton Dickinson (San Jose, CA, USA).

Immunofluorescence and flow cytometry

After culture, THP-1 cells were gently detached with a rubber policeman and washed twice in medium at 4°C. Cell viability, assessed by trypan blue dye exclusion in serum-free medium, was always >95%. Fluorescence staining was then evaluated as described previously [7]. Briefly, cells (2×10^5 in 200 µl of medium) were first incubated for 1 h at 4°C with 200 µl of medium alone (Control), or containing one of the human IgA or IgG preparations, at final concentrations varying between 0.1–5.0 mg·ml^{-1}. After two washes in medium, cells were further incubated for 1 h at 4°C with one of the rabbit FITC-F(ab')$_2$ preparations, all at a final concentration of 3.0 µg·ml^{-1}. After two additional washes in medium, cell-associated fluorescence intensity was then evaluated by flow cytometry with a FACScan flow cytometer (Becton Dickinson). The fluorescence intensity was expressed as log mean channel (LMC). Data are presented in mean±SD of n independent experiments.

Fluorescence studies with My43 MC antibody

The interaction of My43 with THP-1 cells, U-937 cells and human AMs was tested in two separate sets of experiments. For all experiments, cells (2×10^5·ml^{-1}) were initially incubated with 200 µl medium alone, or medium containing 10 µl of control mouse IgM (final concentration 10 µg·ml^{-1}) or with 200 µl of undiluted My43 preparation. After 1 h at 4°C, cells were washed once and, in the first set of experiments, the binding of My43 to cells was evaluated by indirect immunofluorescence. All cells were further incubated with rat FITC-IgM anti-mouse-IgM (20 µg·ml^{-1}), washed once and the cell bound fluorescence was tested by flow cytometry.

In the second set of experiments, the inhibition of IgA-binding to cells by My43 was studied. After an initial incubation with mouse control IgM or My43, cells were washed and incubated for 1 h at 4°C with d-IgA1 (Cr) (5 mg·ml^{-1}), and then for 1 h at 4°C with FITC-F(ab')$_2$ anti-IdA1 (Cr), and finally tested by flow cytometry.

RNA extraction

After culture for 24 h in medium in the presence or absence of 10^{-8} M PMA, THP-1 and U-937 (10^8 cells) were harvested by gentle scraping and immediately processed for RNA extraction following the guanidinium thiocyanate/cesium chloride (CsCl) procedure [28]. Human AMs, shortly after BAL collection, were processed similarly. Briefly, cells were initially washed twice in phosphate-buffered saline and lysed in the guanidinium thiocyanate buffer. The cell lysate was layered on the CsCl solution and ultracentrifuged in a Beckman SW40 rotor, for 18 h at 30,000 rpm. The pellet was resuspended in water and then mixed with a phenol-chloroform-isoamylic alcohol solution, and centrifuged (13,000 rpm, 5 min) in order to remove the proteins. The RNA was purified by means of several precipitations in absolute ethanol. After drying, the RNA was resuspended in water and the concentration was measured by optical density at 260 nm.

Reverse transcriptase (RT) and polymerase chain reaction (PCR)

Complementary deoxyribonucleic acid (cDNA) was synthesised from 3 µg total RNA in a final volume of

20 µl processed by RT [29]. The PCR was performed with 3 µl of cDNA using *Thermolyticus aquaticus* (Taq) DNA polymerase (Perkin Elmer Cetus). Selected primers for a 381 base sequence of the cDNA, encoding for the first extracellular domain of the FcαR in U-937 cells [30], were used for PCR. The base sequences in the primers are as followed: 1) 5'-CAG GAA GGG GAC TTT CCC ATG-3'; and 2) 5'-TGA GCT GCA CGT GAG GGA AAT-3'. After 30 amplification cycles comprising denaturation (1 min, 94°C), annealing (2 min, 59°C) and extension (3 min, 72°C), the products were separated by minigel electrophoresis (1.5% agarose in Tris-acetate-ethylenediamine tetra acetic (EDTA) buffer) with 0.5 µg·ml^{-1} ethidium bromide.

Statistics

Comparisons between groups of values were normally distributed and tested for significance using a paired t-test; p-values <0.05 were considered significant.

Results

Specificity controls. Initial studies designed to evaluate the specificity of the various FITC-F(ab')$_2$ preparations when incubated with THP-1 cells, showed that after incubation with only FITC-F(ab')$_2$ anti-Iso-IgA or with one of the three FITC-F(ab')$_2$ anti-Id, the THP-1 cells displayed similar log mean channels (LMC) of fluorescence intensity, as compared to THP-1 cells incubated only in medium. Moreover, THP-1 cells preincubated with PC IgG at 2.0 mg·ml^{-1} before exposure to the various FITC-F(ab')$_2$ probes, did not display a significant increase in their fluorescence (data not shown).

The specificity of the three different FITC-F(ab')$_2$ anti-Id was also demonstrated by their absence of reactivity toward THP-1 cells preincubated with all non-Id IgA preparations (PC d-IgA1, sIgA) and non-matching IgA-Id (table 1).

IgA binding studies. The fluorescence intensity after binding of MC m-IgA on THP-1 cells was always weaker than that of MC d-IgA or p-IgA at the same concentration, for all IgA preparations tested, using both matching anti-Id probes and the anti-Iso-IgA probe, and always in comparison with the same type of control, *i.e*. THP-1 cells preincubated only with medium and not with MC IgA.

As shown in table 1, using matching anti-Id probes, preincubation of cells with MC m-IgA1 or MC m-IgA2 at 2.0 mg·ml^{-1} induced significant but moderate increases in their LMC (116±22 *vs* 85±4 (Control) for MC m-IgA1(Cl) (n=4), and 105±12 *vs* 83±5 (Control) for MC m-IgA2(Pa) (n=3)). Similar moderate increases in LMC were observed for these MC m-IgA samples using the anti-Iso-IgA probe (table 2). When THP-1 cells were preincubated with MC d-IgA1(Cr) or p-IgA1(Cl) and p-IgA2(Pa), also at 2.0 mg·ml^{-1}, using matching anti-Id probes and the same type of controls as for m-IgA, the LMC observed increased significantly compared to m-IgA (table 1 and fig. 1a and b). Also, with the anti-Iso-IgA probe, the same MC, p-IgA1(Cl) and p-IgA2(Pa) induced larger LMC rises than their m-IgA forms (table 2).

In addition, PC d-IgA1 (0.5 mg·ml^{-1}), and PC sIgA (2.0 mg·ml^{-1}), also elicited significant increases in LMC, using the anti-Iso-IgA probe ((126±4 (n=3) for PC d-IgA1 (fig. 1d), and 153±27 (n=7) for PC sIgA (fig. 1c)). These increases were also larger than those induced by the various MC m-IgA preparations (table 2).

The concentration dependence of the rise in LMC of THP-1 cells preincubated for 1 h at 4°C with various forms of IgA is illustrated in figure 2, with a plateau reached for IgA concentrations above ±2 mg·ml^{-1}. Similar results were obtained for preincubations of 1 h at 37°C (not shown).

Binding of Fcα fragment. The binding of IgA through its Fcα fragment was confirmed by preincubation of THP-1 cells with the Fcα from MC m-IgA1(Cl) at 0.5 mg·ml^{-1}, followed by incubation with the FITC-F(ab')$_2$ anti-Iso-IgA. This procedure induced a significant rise in LMC from the control value of 87 to 169±12 (n=3) (table 2). Another type of control employed preincubation of the THP-1 cells with the Fcα fragment at the same concentration, but used the FITC-F(ab')$_2$ anti-IdA1(Cl) FITC-probe, which should not react with the Fcα fragment of IgA1(Cl). Indeed, under such conditions, there was virtually no increase in LMC (94±7 (n=3)).

PMA, retinoic acid and calcitriol stimulation. THP-1 cells were exposed to a broad range of concentrations (10^{-12} to 10^{-5}) of the three stimuli. Only PMA at an optimal concentration of 10^{-8} M induced a significantly

Table 1. – Specificity of the FITC-F(ab')$_2$ anti-Id probes

Preincubation	FITC-F(ab')$_2$ probes		
	anti-IdA1 (Cl)	anti-IdA1 (Cr)	anti-IdA2 (Pa)
Medium	85±4*	85±5	83±5
Id IgA1 (Cl)			
m-	116±22°	-	-
p-	293±18°	104±20	98±7
Id IgA1 (Cr)			
d-	93±7	215±16†	94±5
Id IgA2 (Pa)			
m-	-	-	105±12^{+}
p-	80±8	-	144±18^{+}

Cells are first preincubated with medium or one of the Id IgA preparations at 2 mg·ml^{-1} and then with the FITC-F(ab')$_2$ anti-Id. *: all data of fluorescence intensity (LMC) represent the mean±SD of at least four separate experiments (n=4), except for m-Id IgA2 (n=3); $^{\circ}$: p<0.05, compared to FITC-F(ab')$_2$ anti-IdA1 (Cl) alone; †: p<0.05, compared to FITC-F(ab')$_2$ anti-IdA1 (Cr) alone; $^{+}$: p<0.05, compared to FITC-F(ab')$_2$ anti-IdA2 (Pa) alone. FITC: fluorescein isothiocyanate; Id: idiotype; m-: monomeric; p-: polymeric; d-: dimeric; IgA: immunoglobulin A; LMC: log mean channel.

Table 2. – Binding of various IgA preparations to THP-1 cells

	PC		MC				
Medium	d-IgA1 0.5 mg·ml^{-1}	sIgA 2 mg·ml^{-1}	m-IgA1 (Cl) 2 mg·ml^{-1}	p-IgA1 (Cl) 2 mg·ml^{-1}	Fc m-IgA1 (Cl) 0.5 mg·ml^{-1}	m-IgA2 (Pa) 2 mg·ml^{-1}	p-IgA2 (Pa) 2 mg·ml^{-1}
87±6 n=9	126±4 n=3	153±27 n=7	113±6 n=4	270±13* n=5	169±12 n=3	100±8 n=3	120±15* n=5

The binding of various IgA preparations to THP-1 revealed by FITC-F(ab')$_2$ anti-Iso-IgA after incubation with the respective IgA. Data of fluorescence intensity (LMC) are presented as the mean±SD of n independent experiments. All IgA preparations tested induced a significant increase in fluorescence compared to the control (medium alone). sIgA: secretory IgA; PC: polyclonal; MC: monoclonal. For further abbreviations see legend to table 1. *: $p<0.05$ compared to the corresponding m-IgA.

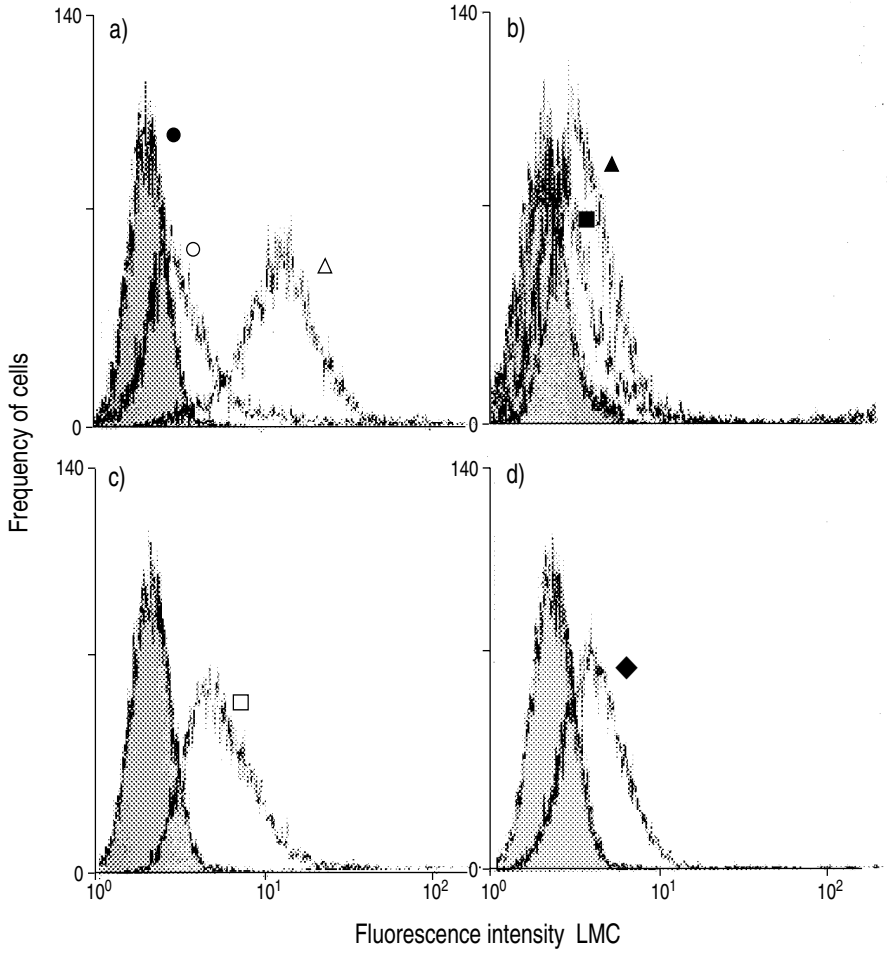

Fig. 1. – IgA-binding to THP-1 cells: flow cytometric analysis of the cell-bound fluorescence expressed in LMC. In each panel, the shaded area represents the autofluorescence of THP-1 cells (in the absence of FITC-probes), and the histogram corresponding to the fluorescence obtained in the presence of control non-anti-IgA FITC-F(ab')$_2$ (●) is similar to the autofluorescence. a) MC m-IgA1 (Cl) (2.0 mg·ml^{-1}) induced a limited increase in fluorescence (histogram ○) compared to MC p-IgA1 (Cl) at the same concentration (histogram Δ), both revealed by the anti-Id-(Cl) FITC-F(ab')$_2$. b) a similar profile of fluorescence is observed for IgA2, with a higher binding of MC p-IgA2 (histogram ▲) compared to its monomeric form (histogram ■), both at 2.0 mg·ml^{-1} and revealed by the anti-Id-(Pa) FITC-F(ab')$_2$. c) binding of sIgA (2.0 mg·ml^{-1}) to THP-1 cells with anti-Iso-IgA FITC-F(ab')$_2$ (histogram ❑). d) binding of PC d-IgA1 (0.5 mg·ml^{-1}) using the anti-Iso-IgA FITC-probe (histogram ◆). IgA: immunoglobulin A; LMC: log mean channel; FITC: fluorescein isothiocyanate; MC: monoclonal; m-: monomeric; p-: polymeric; Id: idiotype; sIgA: secretory IgA; PC: polyclonal; d-dimeric.

($p<0.05$) greater binding of IgA (p-IgA1(Cl), 0.5 mg·ml^{-1}) with a LMC of 285±16 (n=4), using anti-IdA1(Cl) FITC-F(ab')$_2$ as probe, compared to binding observed in nonstimulated cells (LMC=187±11). Retinoic acid and calcitriol had no significant effect on IgA-binding to THP-1 cells.

THP-1 cell differentiation. To evaluate the cell differentiation at the level of the cell surface markers, the presence of CD14 was tested on THP-1 cells, before and after incubation with PMA, retinoic acid and calcitriol, at concentrations up to 10^{-6} M, and for times varying between 1–72 h. The CD4 marker, known to be constitutively present on

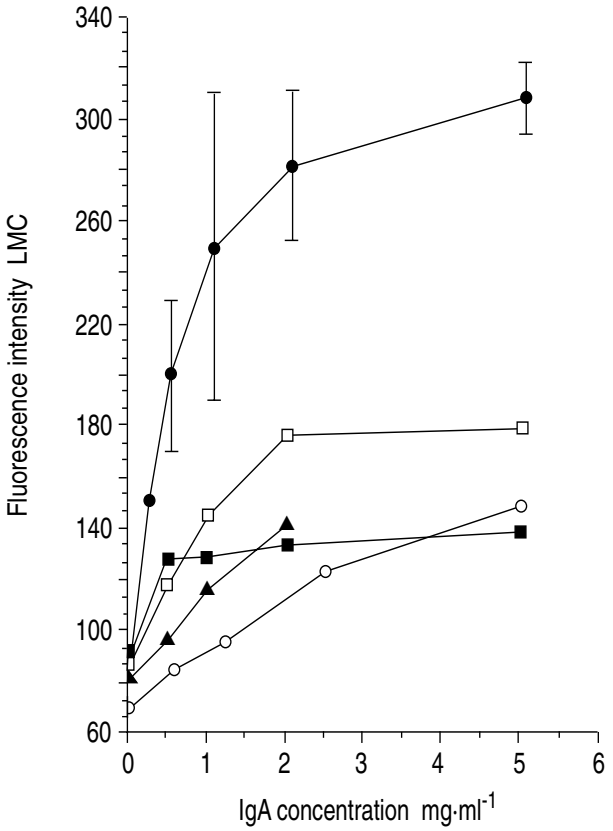

Fig. 2. – Effect of various IgA concentrations of IgA-binding to THP-1 cells. Curves were obtained, respectively, for MC p-IgA1 (Cl) (●) (mean±SD of four experiments); MC m-IgA1 (Cl) (○); MC p-IgA2 (□); MC m-IgA2 (■); and PC sIga (▲). The matching anti-Id FITC-F(ab')$_2$ were used for MC m- and p-IgA1 (Cl) and for MC m- and p-(IgA2 (Pa), and the anti-Iso-IgA FITC-F(ab')$_2$ for Pc sIgA. For abbreviations see legend to figure 1.

THP-1 cells, was used as a positive control and was present similarly on unstimulated and retinoic acid- and calcitriol-stimulated THP-1 cells, as demonstrated by a similar increase in fluorescence induced by the FITC-anti-CD4 with a LMC=118±8 *vs* 83±9 in control (n=4). As reported previously, PMA (10^{-9} to 10^{-6} M) rapidly diminished CD4 expression on THP-1 [31].

By contrast, only calcitriol (optimal concentration 10^{-7} M, optimal time 24 h) could induce the expression of CD14 on THP-1 cells, as revealed by the FITC-anti-CD14, with a LMC of 321±60 (n=4), compared to non-stimulated or PMA- and retinoic acid-stimulated THP-1 cells in presence of the same probe (LMC=83±9).

Studies with anti-FcαR MC IgM antibody My43. Initial studies evaluated the binding of My43 to THP-1 cells using indirect fluorescence with a rat MC FITC-IgM anti-mouse-IgM. The control consisted of an incubation with a nonspecific mouse IgM followed by the same rat FITC-probe. Under control conditions, the LMC was 81±8 (n=4). As shown in figure 3a, no increase of LMC was observed when My43 was substituted for control mouse IgM, with a LMC of 94±11. A similar profile was observed with normal AMs collected by BAL. Thus, the LMC of AMs in the presence of My43 was 120±16, whilst that in the presence of control mouse IgM was 135±13 (n=4). By contrast, U-937 cells preincubated with My43 before staining with the rat anti-mouse-IgM FITC-probe displayed a significant increase in LMC (137±19), when compared to U-937 cells incubated with control mouse IgM (LMC=86±11 (n=4)) (fig. 3b).

Similarly, using FITC-F(ab')$_2$ anti-IdA1(Cr) as probe, My43 could inhibit the binding of MC d-IgA1 (Cr) (0.5 mg·ml^{-1}) to U-937 cells, with LMC of 147±16 and 220±18, respectively, in the presence and absence of preincubation

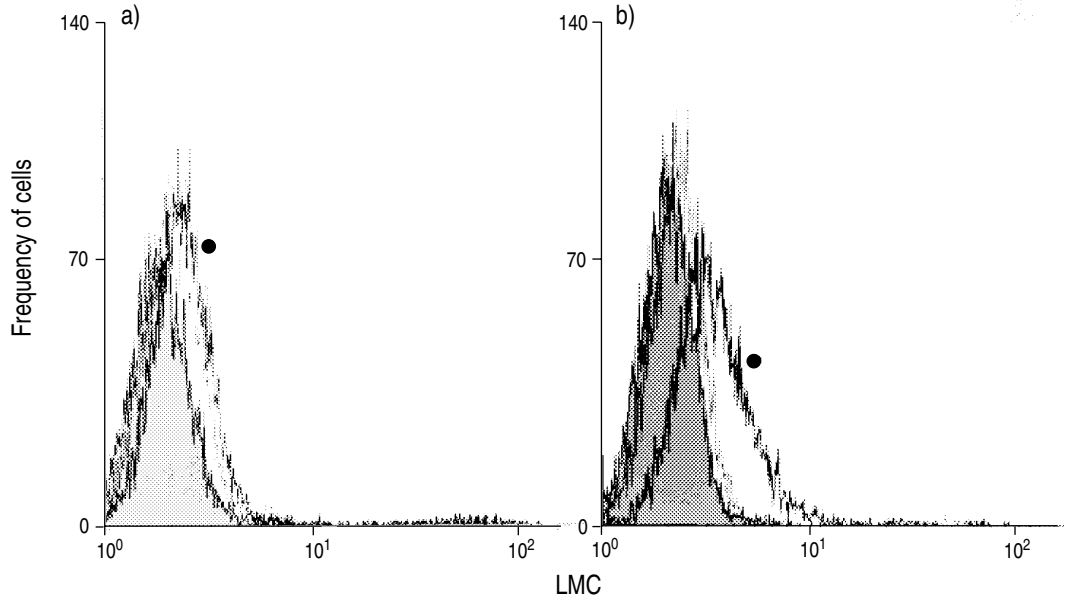

Fig. 3. – My43-binding studies with: a) THP-1 cells; and b) U-937 cells. The shaded area corresponds to the autofluorescence. The pale histogram (control) represents the fluorescence observed in the presence of control mouse IgM and rat FITC-IgM against mouse IgM, and the wider histogram (●) illustrates the fluorescence in the presence of My43 and the rat FITC-probe. My43 significantly increased the cell fluorescence compared to the control histogram only in U-937 cells. IgM: immunoglobulin M; FITC: fluorescein isothiocyanate.

Table 3. – Effect of My43 on IgA-binding to THP-1, U-937 and alveolar macrophages (AMs)

	Control mouse IgM + medium +FITC-F(ab')$_2$ anti-IdA1 (Cr)	Control mouse IgM +Id IgA1 (Cr) 0.5 mg·ml^{-1} +FITC-F(ab')$_2$ anti-IdA1 (Cr)	My43 - IgM +Ig IgA1 (Cr) 0.5 mg·ml^{-1} +FITC-F(ab')$_2$ anti-IdA1 (Cr)
THP-1	80*	175	186
U-937	82	220	147^{+}
AMs	128	290	281

Cells are initially incubated with mouse control IgM or My43, washed and further incubated with MC d-IgA1 (Cr) and IgA-binding is revealed with FITC-F(ab')$_2$ anti-Id (Cr) as described in Methods. *: all data expressed in LMC represent mean values of four independent experiments; $^{+}$: significantly lower IgA-binding than that obtained with control IgM. IgM: immunoglobulin M. For further abbreviations see legend to tables 1 and 2.

with My43 (n=4) (table 3). No inhibition of binding of d-IgA1(Cr) was observed for THP-1 cells and for human AMs (no change in LMC in the presence or absence of My43 (n=4)), as shown in table 3. Similar data were obtained with d-IgA1 (Cr) at 2.0 mg·ml^{-1}.

Molecular biological studies. As illustrated in figure 4, RNA encoding the FcαR was detected in THP-1, U-937 cells and human AMs. However, whilst the expression of RNA for β-actin was similar in the three cell types, the expression of RNA for the FcαR appeared to be lower in THP-1 (both unstimulated and PMA (10^{-8} M) stimulated) and human AMs than in unstimulated and PMA (10^{-8} M) stimulated U-937 cells.

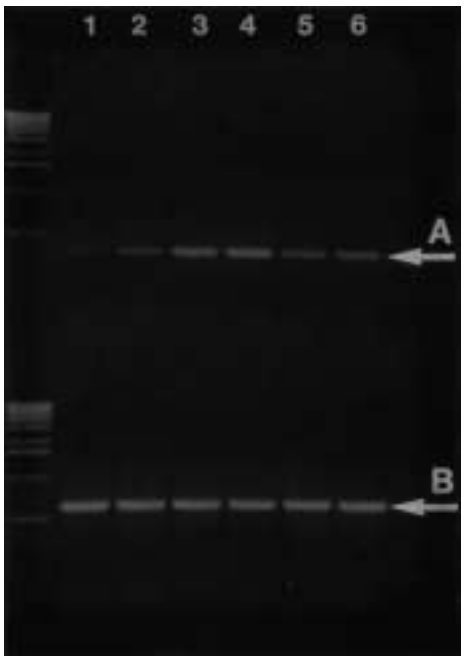

Fig. 4. – Expression of RNA for FcαR in: unstimulated THP-1 (lane 1); PMA (10^{-8} M) stimulated THP-1 (lane 2); unstimulated U-937 (lane 3); PMA-stimulated U-937 (lane 4); and two pools of human alveolar macrophages from normal donors (lanes 5 and 6), with the standard base pairs scale on the left. The cDNA sequence selected, encoding for the FcαR, migrates as a 381 b.p band (arrow A). As shown, the expression appears weaker in human AMs and in unstimulated and PMA-stimulated THP-1 when compared to U-937, while β-actin mRNA expression (arrow B) is similar for all cells examined. RNA: ribonucleic acid; FcαR: immunoglobulin A Fc receptor; PMA: phorbol myristate acetate; cDNA: complimentary deoxyribonucleic acid; mRNA: messenger RNA.

Discussion

The presence of FcαR has been demonstrated on the surface of several human mononuclear and polynuclear phagocytic cells or cell lines [5–8, 10]. We have reported the presence of cytophilic IgA and FcαR on human AMs [7]. Considering the substantial amount of IgA in the bronchial epithelium lining fluid and the key role of human AMs in the respiratory tract, the interplay between these two immune components could provide additional protective mechanisms [1]. Several drawbacks prevent an easy access to the evaluation of the FcαR on human AMs. These include the presence of numerous other surface receptors and ligands, the difficulty in obtaining pure AM populations from the lung, their heterogeneity and phenotypic difference among donors. To continue the investigation of FcαR on human mononuclear phagocytes, we therefore decided to use a leukaemia cell line sharing some characteristics with tissue macrophages, such as phagocytosis, cytokine release and the presence of surface Fcγ-receptors.

THP-1 is a macrophage-like cell line, obtained from a child with a promyelocytic leukaemia, expressing a phenotype suggesting a blockade at a late stage of maturation. Here, we observe that unstimulated THP-1 cells constitutively express FcαR. These bind MC IgA (two IgA1 and one IgA2), both monomeric and polymeric forms. PC serum-derived d-IgA1 and milk sIgA also bound to THP-1 cells. Although the higher increase in fluorescence obtained with p-IgA suggests that the putative FcαR on THP-1 is a low affinity receptor, we cannot rule out that this higher fluorescence intensity could be related to an amplification effect of the anti-IgA, recognizing more epitopes on p-IgA than on m-IgA. However, our previous studies on human and rat AMs, using both direct and indirect fluorescence, also demonstrated higher increase in fluorescence with p-IgAs as compared to m-IgAs [7, 32]. The observation that the IgA-binding was concentration-dependent, usually with optimal binding obtained at concentrations greater than 2 mg·ml^{-1}, illustrates that relatively high concentrations are required to reach saturation binding, suggesting a rather low density of receptors on the cell surface and/or a relatively low affinity for IgA.

In a previous study using U-937 cells, IgA in medium at 37°C for various periods of time was claimed to induce expression of FcαR on the cell surface [33]. In

the present study with THP-1 cells, we did not observe a difference between bindings obtained at 4° and 37°C with the same IgA concentration for incubations ranging from 30 min to 12 h (data not shown). This suggests that higher IgA concentrations increase IgA-binding to THP-1 cells, rather than induce the expression of new FcαR on the cell surface. This also suggests that the FcαR is not internalized during this incubation at 37°C, a finding in contrast with our observation in human AMs [7].

Although PMA (10^{-8} M) significantly increased the IgA-binding to THP-1 cells, this increase was not as great as that described for other promyelocytic cell lines, in particular HL-60 and U-937 [12, 33]. This could be related to the cell line, its stage of maturation, the stimuli used, the high constitutive expression of FcαR on THP-1 cells, or to a combination of several of these factors. After PMA stimulation, THP-1 cells experience morphological (and phenotypical) changes and increase their adherence to culture dishes, as reported previously [15]. Such changes could also interfere with IgA-binding to the cell surface. Among the different stimuli used, only calcitriol could induce THP-1 differentiation, as assessed by the expression of CD14. In these conditions, however, no increase in IgA-binding could be detected, supporting the observation that calcitriol-induced differentiation of THP-1 cells did not influence FcαR expression.

Recently, the expression of Fc-γ-receptors was investigated in THP-1 cells. FcγR-I and FcγR-II but not FcγR-III were constitutively expressed by these cells, and among a series of stimuli, only γ-interferon (γ-IFN) could substantially increase IgG-binding to THP-1 cells. As observed for FcαR, the FcγR expression on THP-1 cells appeared to be independent of cell differentiation [34].

My43 is a MC mouse IgM, which recognizes the FcαR on human peripheral blood monocytes and the promyelocytic cell lines U-937 and HL-60 [12]. Moreover, My43 could inhibit IgA rosette formation and IgA-mediated phagocytosis in monocytes [12]. We have confirmed both the binding of My43 to U-937 cells and the inhibition of IgA-binding to U-937 cells by My43. In the present study, however, My43 did not bind to THP-1 cells and was unable to block their IgA-binding. Similar data were obtained with human AMs from normal subjects. This might suggest that the FcαR on THP-1 cells and human AMs differs from the FcαR on U-937 cells and monocytes, or that the epitope of the FcαR recognized by My43 is either absent or masked on THP-1 cells and normal human AMs. My43 was used to isolate a clone from a U-937 cDNA library directing the expression of the myeloid FcαR [30]. This cDNA encodes a 30 kDa protein with IgA-binding specificity and homologies to several other Fc-receptors. This 30 kDa protein represents the polypeptide part of the previously reported heavily glycosylated ≈60 kDa FcαR glycoprotein isolated from human mononuclear and polymorphonuclear leucocytes [35]. Our molecular biological studies demonstrate that human AMs and THP-1 cells do express RNA encoding at least part of the FcαR, but apparently at a lower level compared to U-937 cells. Moreover, PMA stimulation appears to increase the expression level of the gene in THP-1 but not in U-937 cells.

In addition to My43, several monoclonal antibodies against FcαR present on human phagocytes have been characterized [36]. Using these antibodies, MONTEIRO *et al*. [36] recently reported a similar protein core, but differences in glycosylation, between the FcαR of eosinophils compared to neutrophils. In collaboration with these authors, we are at present testing these monoclonal antibodies to further evaluate the putative difference in FcαR on THP-1 and AM.

In conclusion, THP-1 cells constitutively bind IgA and express FcαR on their surface. The profile of IgA-binding to THP-1 cells is grossly similar to that observed for human AMs, with binding of Fcα fragments and all IgA preparations tested, including monoclonal and polyclonal, monomeric and polymeric forms of both subclasses and secretory IgA. By contrast with U-937 cells and peripheral blood monocytes, THP-1 cells and human AMs did not bind My43. The latter observation could, in part, be explained by a lower expression of the FcαR RNA in human AMs and THP-1 cells compared to U-937 cells. Finally, THP-1 cells represent a reasonable model for further investigation of the structural and functional aspects of FcαR of mature tissue macrophages.

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