

HUMAN RECOMBINANT INTERLEUKIN 4 INDUCES FcεR2/CD23 ON NORMAL HUMAN MONOCYTES

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Two types of receptors for the Fc portion of human IgE (FcεR) have been identified on the surface of human cells. These two types of receptors differ in their affinity for IgE as well as in their cellular distribution and function (1). Type 1 FcεR (FcεR1) show high affinity (10^9 /M) for IgE and are detectable on mast cells and basophils (2). The reaction between allergens and IgE bound to FcεR1 induces degranulation of basophils and mast cells, with release of chemical mediators responsible for the clinical manifestations of allergy (3). In contrast, type 2 FcεR (FcεR2) bind IgE with a lower affinity (10^7 – 10^8 /M) and are present on monocytes, macrophages, B lymphocytes, platelets, and eosinophils (4). The binding of IgE to FcεR2 on monocytes, eosinophils, and platelets activates a variety of effector functions in these cells (3, 5, 6).

It is well known that the frequency of human peripheral blood monocytes (Mo)¹ bearing FcεR2 is increased in atopic subjects, particularly in the presence of high serum IgE levels, while it is negligible in normal subjects (3). In addition, it has been recently reported that Mo bearing cytophilic IgE infiltrate into the skin lesions of patients with atopic dermatitis (7). The role of FcεR2⁺ Mo in the pathogenesis of allergic disorders is, however, still poorly understood. Binding of IgE to FcεR2 sensitizes rodent peritoneal (8) and alveolar (9) macrophages to the release of leukotrienes and arachidonic acid upon exposure to allergen.

The signals that are able to induce the expression of FcεR2 on Mo, however, have not been defined. In the present study, we have examined the ability of a variety of recombinant lymphokines and monokines to induce on human normal peripheral blood Mo the expression of FcεR2, as detected by IgE binding. In parallel, we have investigated the ability of the same agents to induce the expression on Mo of the CD23 antigen, as detected by mAb binding. CD23 has been described as a B cell-specific activation antigen (10) and more recently has been shown to be identical to the B cell FcεR2 (11, 12) and to the Blast-2 antigen expressed by B cells upon activation by a variety of stimuli (13, 14). Our data indicate that the expression of FcεR2/CD23 by normal human Mo can be spe-

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¹Abbreviations used in this paper: BSF, B cell stimulatory factor; GAHE, goat anti-human IgE; GAMIG, goat anti-mouse IgG; GM-CSF, granulocyte/macrophage colony stimulating factor; IF, immunofluorescence; MCF, median channel fluorescence; Mo, monocytes; PE, phycoerythrin.

cifically induced by IL-4/B cell stimulatory factor 1 (BSF-1), but not by IL-1, IL-2, IL-3, IL-5, BSF-2, granulocyte/macrophage colony-stimulating factor (GM-CSF), and IFN- γ .

Materials and Methods

Immunoglobulin and Antibodies. Human myeloma IgE (PS) (a kind gift of Dr. K. Ishizaka, Johns Hopkins University, Baltimore, MD) was purified as previously described (15). A FITC-conjugated, affinity-purified goat anti-human IgE(PS) (GAHE) was prepared as previously described (16). The murine mAb 25 raised against the human B cell line RPMI 8866 and specific for CD23/Fc ϵ R2 (11) was kindly provided by Drs. J. Banchereau and J. de Vries, UNICET Laboratory for Immunological Research, Dardilly, France. The mouse anti-human anti-Blast-2 (EBVCS2) mAb (13) was a kind gift of Dr. B. Sugden, McArdle Laboratory for Cancer Research, Madison, WI. Anti-HLA-Dr, anti-Leu-M3 (unconjugated and phycoerythrin (PE)-conjugated) and anti-Leu-16 mAbs, FITC-conjugated goat anti-mouse IgG (GAMIG), and control mAbs of appropriate Ig isotypes were purchased from Becton Dickinson Monoclonal Center (Mountain View, CA). Plt-1, a murine IgM mAb specific for the glycoprotein IIb-IIIa on human platelets, was purchased from Coulter Immunology (Hialeah, FL). A FITC-conjugated goat anti-mouse IgM was obtained from Tago Inc. (Burlingame, CA).

Interleukins. Human rIL-4 was kindly provided by Dr. K. Arai, DNAX Research Institute of Cellular and Molecular Biology (Palo Alto, CA) as supernatants from COS-7 cells transfected with the pcD vector containing the human IL-4 cDNA clone. Mock supernatants from COS-7 cells transfected with an unrelated cDNA clone (mock sup) were used as a negative control. Human rIL-1 β , rIL-2, rIL-3, and rIFN- γ were obtained from Cistron (Pine Brook, NJ), Amgen Biologicals (Thousand Oaks, CA), Genzyme Corporation (Boston, MA), and Biogen Corporation (Cambridge, MA), respectively. Human rGM-CSF and rIL-5 were a kind gift of Dr. S. Clark, Genetics Institute (Cambridge, MA). Human rBSF-2 was a kind gift of Dr. T. Kishimoto, Institute for Cellular and Molecular Biology, Osaka, Japan.

Isolation of Peripheral Blood Mo. PBMCs were isolated from heparinized venous blood of healthy human donors by Ficoll-Hypaque density gradient centrifugation. The cells were then resuspended in RPMI 1640 (M.A. Bioproducts, Walkersville, MD) supplemented with 10% AB⁺ serum (Hazleton Research Products, Lenexa, KA), 2 mM glutamine, 50 μ g/ml streptomycin, and 100 U/ml penicillin (complete medium), and were adhered overnight at 37°C in a 5% CO₂ atmosphere in plastic petri dishes. After removing the nonadherent cells, the dishes were extensively washed with warm medium and then incubated with cold PBS on ice for 15 min. The adherent Mo were subsequently recovered by vigorous pipetting, washed, and resuspended in complete medium at 10⁶ cells/ml. The Mo preparations contained >95% Leu-M3⁺ cells and <3% Leu-16⁺ cells, as detected by immunofluorescence staining. Platelet contamination (10–20%), as assessed by staining with the Plt-1 murine mAb, was detectable in some Mo preparations. Cell viability, as determined by trypan blue exclusion, was always >95%.

Cell Cultures. After preliminary experiments to establish the optimal concentration of each IL, purified Mo (10⁶ cells/ml) were incubated with rIL-4 (50 U/ml), rGM-CSF (1 ng/ml), rIFN- γ (200 U/ml), rIL-1 (10 U/ml), rIL-2 (10 U/ml), rIL-3 (50 U/ml), rIL-5 (50 U/ml), rBSF-2 (100 U/ml), and mock supernatants. After 24–48 h, the cells were washed and analyzed by immunofluorescence to assess the expression of Fc ϵ R2/CD23.

In some experiments, purified Mo (10⁶ cells/ml) were incubated with various concentrations (0.1–100 ng/ml) of PMA (Sigma Chemical Co., St. Louis, MO) with, or without, 10 μ M ionomycin (Calbiochem-Behring Corp., La Jolla, CA).

In control experiments, human platelets (1.5 \times 10⁸/ml) purified from three different normal donors as previously described (6) were incubated with rIL-4 (50 U/ml) for 24 h and subsequently analyzed by flow cytometry for Fc ϵ R2/CD23 expression.

Immunofluorescence (IF) Staining for Fc ϵ R2/CD23. The expression of Fc ϵ R2/CD23 on

Mo incubated with different ILs was evaluated by indirect IF. (a) 0.5×10^6 Mo in staining buffer (RPMI 1640/2.5% FCS, containing 0.01% sodium azide) were incubated with IgE (PS) (10 $\mu\text{g}/\text{ml}$) for 40 min at 4°C. After washing, the cells were incubated for 30 min at 4°C with GAHE-FITC and finally extensively washed. (b) In parallel, Mo were incubated for 40 min at 4°C with appropriate dilutions of mAb 25 or anti-Blast-2 mAb and, after washing, with GAMIG-FITC for 30 min at 4°C. As a control, an unrelated mouse IgG mAb was used, followed by GAMIG-FITC, under the same conditions. The percentage of Mo binding IgE and the CD23-specific mAbs was evaluated by a FACS cell analyzer (Becton Dickinson & Co.). Background staining (always <5%) was subtracted from the experimental values. To evaluate the expression of HLA-Dr antigens, purified Mo were stained with anti-HLA-Dr mAb followed by GAMIG-FITC, and median channel fluorescence (MCF) was determined. MCF, a measure of fluorescence intensity, correlates well with antigen density (17). MCF of the control was subtracted from the MCF of each sample.

For binding inhibition assays, 0.5×10^6 FcεR2/CD23⁺ U937 cells were preincubated with medium, anti-Blast-2 mAb (1 $\mu\text{g}/\text{ml}$), or a control IgG1 murine mAb (anti-HLA-DP, 2.5 $\mu\text{g}/\text{ml}$) for 60 min at 4°C. The cells were then washed twice and incubated for 40 min at 4°C with mAb 25-FITC (1 $\mu\text{g}/\text{ml}$) or with IgE(PS) (1 $\mu\text{g}/\text{ml}$) followed by GAHE-FITC. After extensive washing, the percentage of cells binding the second ligand was assessed as described above. The background value was determined by the percentage of cells stained by a FITC-conjugated control mAb of IgG1 isotype or by GAHE-FITC.

For two-color IF staining, purified Mo (0.5×10^6 cells/ml) in staining buffer were incubated with appropriate dilutions of mAb 25-FITC and anti-Leu-M3-PE for 40 min at 4°C. After extensive washing, the percentage of double-staining cells was evaluated by FACS analysis. Unrelated fluorochrome-conjugated murine mAbs of the appropriate IgG isotype were used as controls. Background staining was subtracted from the experimental values.

Surface Iodination and Immunoprecipitation. Purified Mo (20×10^6 cells) were incubated with medium or rIL-4 (50 U/ml) for 48 h. The cells were then washed, suspended in 0.2 ml of PBS, and surface iodinated (1 mCi ¹²⁵I-Na; New England Nuclear, Boston, MA) by the lactoperoxidase method (18). FcεR2/CD23⁺ U937 and RPMI 8866 cells were treated under the same conditions. The cell membranes were solubilized at 4°C in 0.3 ml of lysis buffer containing 1.5% NP-40 in PBS and proteolytic enzyme inhibitors: 1 mM PMSF (Sigma Chemical Co.), 1mM EDTA, 10 mM iodoacetamide, and 1 $\mu\text{g}/\text{ml}$ of leupeptin, antipain, chymostatin, and pepstatin (Sigma Chemical Co.). Cell lysates were clarified by centrifuging at 100,000 g for 20 min at 4°C and were incubated overnight with 10 μl of a 10% suspension of *Staphylococcus aureus* organisms (IgG-sorb, The Enzyme Centre Inc., Malden, MA). This was followed by three absorption cycles, each consisting of a 2-h incubation with 30 μl of protein A-Sepharose 4B beads (Pharmacia Fine Chemicals, Piscataway, NJ) coupled to normal mouse IgG (CooperBiomedical, Inc., Malvern, PA). Immunoprecipitation was then performed by incubating the clarified cell lysates with mAb 25 (10 μg) bound to protein A-Sepharose 4B (20 μl). As a negative control, the cell lysates were immunoprecipitated with 10 μg of normal mouse IgG coupled to protein A-Sepharose 4B. In some experiments, immunoprecipitation was performed with IgE(PS) coupled to Sepharose 4B (10 mg/ml). The beads were extensively washed with lysis buffer and then eluted by boiling for 5 min in electrophoresis sample buffer. The samples were analyzed by SDS-PAGE according to the method of Laemmli (19), and autoradiographed (Kodak diagnostic film XAR-5).

Results

rIL-4 Induces the Expression of FcεR2/CD23 on Normal Human Mo. To identify soluble signals able to induce and/or upregulate the expression of FcεR2 on normal human Mo, purified Mo were incubated with a variety of rILs. 24–48 h later, the Mo were assayed by flow cytometry for the expression of FcεR2 using

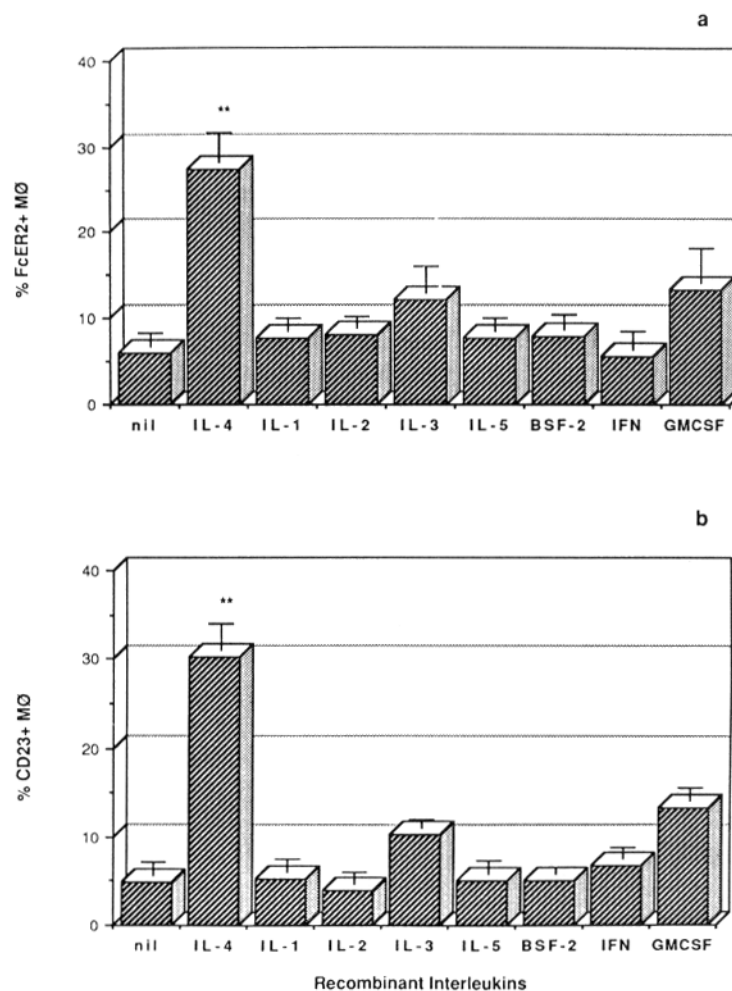


FIGURE 1. Human rIL-4 induces the expression of FcεR2/CD23 on normal human Mo. Purified human Mo were incubated for 24–48 h with rIL-4 (50 U/ml), rIL-1 (10 U/ml), rIL-2 (10 U/ml), rIL-3 (50 U/ml), rIL-5 (50 U/ml), rBSF-2 (100 U/ml), rIFN-γ (200 U/ml), rGM-CSF (1 ng/ml), and mock sups. The Mo were then assayed by flow cytometry for the expression of FcεR2 (using soluble IgE followed by GAHE-FITC [a]) and CD23 (using mAb 25 followed by GAMIG-FITC [b]). The data represent the mean ± SE of 11 experiments. Statistical analysis was performed by a two-tailed Student's *t* test. (**)*p* < 0.001.

soluble IgE and a FITC-conjugated affinity-purified GAHE. The results of 11 experiments are shown in Fig. 1 *a*. The baseline expression of FcεR2 on normal Mo was negligible (<6%). Incubation with rIL-4 (50 U/ml), however, induced a significant (*p* < 0.001) increase in the expression of FcεR2. Induction of FcεR2 by rIL-4 was specific, since a mock COS-7 transfection supernatant had no effect on FcεR2 expression (data not shown). In contrast, no significant increase in FcεR2 was observed when Mo were incubated with rIL-1 (10 U/ml), rIL-2 (10 U/ml), rIL-3 (50 U/ml), rIL-5 (50 U/ml), rBSF-2 (100 U/ml), rIFN-γ (200 U/ml), rGM-CSF (1 ng/ml), either alone (Fig. 1 *a*) or in various combinations (data

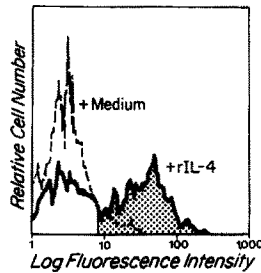


FIGURE 2. Flow cytometry analysis of rIL-4-induced FcεR2/CD23 expression on normal human Mo. Purified human Mo incubated for 24 h with medium (---) or rIL-4 (50 U/ml) (—) were assessed by IF for the expression of FcεR2/CD23, as detected by the CD23-specific mAb 25 followed by GAMIG-FITC. The shaded area represents the FcεR2/CD23⁺ cell population.

not shown). No synergism was observed between IL-4 and other ILs (data not shown). Fig. 2 shows a representative example of the level of rIL-4-induced FcεR2 expression observed by flow cytometry.

In Fig. 1 *b*, Mo were incubated with the same combinations of ILs. In these experiments, however, the expression of CD23 was assessed by IF using mAb 25, which recognizes CD23/FcεR2 on RPMI 8866 B cells (11). A comparison of Fig. 1 *a* and 1 *b* clearly indicates that the expression of FcεR2 (as assessed by soluble IgE binding) and CD23 (as assessed by anti-CD23 mAb binding) followed an identical pattern.

The same pattern was also obtained when the induction of the Blast-2 antigen (as detected by anti-Blast-2 mAb binding) was studied (data not shown). The Blast-2 antigen has to date been considered a B cell-specific activation marker expressed by B cells upon stimulation with EBV or mitogens (13, 14). To further investigate the relationship between FcεR2/CD23 and Blast-2 on Mo, we examined the ability of the anti-Blast-2 mAb to inhibit the binding of IgE and/or mAb 25 to the human promonocytic cell line U937. U937 cells constitutively express FcεR2/CD23 and Blast-2 at high levels (82 ± 1 and $88 \pm 1\%$, respectively, as assessed by IF). As shown in Table I, anti-Blast-2 mAb strongly inhib-

TABLE I
Anti-Blast-2 mAb Inhibits the Binding of mAb 25 and IgE to U937 Cells

Preincubation with:	Second ligand	Positive cells	Inhibition
		%	%
Medium	mAb 25-FITC	75 ± 3	—
Anti-Blast-2	mAb 25-FITC	6 ± 1	92
Control mouse			
IgG1	mAb 25-FITC	68 ± 2	10
Medium	IgE+GAHE-FITC	84 ± 1	—
Anti-Blast-2	IgE+GAHE-FITC	20 ± 2	71
Control mouse			
IgG1	IgE+GAHE-FITC	70 ± 3	17

0.5×10^6 U937 cells in staining buffer were preincubated with medium, anti-Blast-2 mAb (1 $\mu\text{g}/\text{ml}$), or a control IgG1 murine mAb (anti-HLA-DP, 2.5 $\mu\text{g}/\text{ml}$) for 60 min at 4°C. After washing, the cells were then incubated for 40 min at 4°C with mAb 25-FITC (1 $\mu\text{g}/\text{ml}$) or with IgE(PS) (1 $\mu\text{g}/\text{ml}$) followed by GAHE-FITC. The table shows the percentage of positive cells subtracted for the background (percentage of cells stained by a FITC-conjugated control mAb of IgG1 isotype or by GAHE-FITC). The data represent the mean \pm SE of three experiments.

ited the binding of mAb 25 (92% inhibition) and IgE (77% inhibition) to U937 cells. No significant inhibition was detectable using a control IgG1 mAb. These data indicate that the Fc ϵ R2/CD23 and Blast-2 antigens expressed by human Mo are closely related, if not identical.

We next wished to confirm that the IL-4-induced Fc ϵ R2/CD23 was expressed on Mo, and not on contaminating B cells and/or platelets. To this purpose, purified Mo incubated with rIL-4 were analyzed by two-color IF for simultaneous reactivity with mAb 25-FITC and anti-Leu-M3-PE. In four experiments, the percentage of cells stained with both the anti-Leu-M3 and mAb 25 antibodies rose from $4 \pm 1\%$ in control cultures to $49 \pm 3\%$ in the rIL-4-treated cultures. Furthermore, rIL-4 had no effect on Fc ϵ R2/CD23 expression (as assessed by flow cytometry using soluble IgE or CD23-specific mAbs) by purified human platelets from three different donors (data not shown). These data indicate that Mo indeed express Fc ϵ R2/CD23 upon stimulation with rIL-4.

Kinetics of the IL-4-induced Expression of Fc ϵ R2/CD23 on Mo. The time course of Fc ϵ R2/CD23 expression was assessed by flow cytometry at various times after the addition of human rIL-4 (50 U/ml). As shown in Fig. 3, Fc ϵ R2/CD23 was still absent after 8 h of incubation with rIL-4, peaked 24 h after the addition of rIL-4, and was still clearly detectable after 72 h of incubation with rIL-4. The same kinetics pattern was obtained monitoring the induction of the Blast-2 epitope (data not shown).

Immunoprecipitation of Fc ϵ R2/CD23 from Human Mo. To confirm the induction of the CD23 antigen on normal human Mo upon stimulation with rIL-4, normal human Mo (preincubated for 48 h with medium or rIL-4 [50 U/ml]) were surface labeled with ^{125}I , then lysed and immunoprecipitated with mAb 25. As shown in Fig. 4, incubation with rIL-4 (Fig. 4, lane 3) induced the appear-

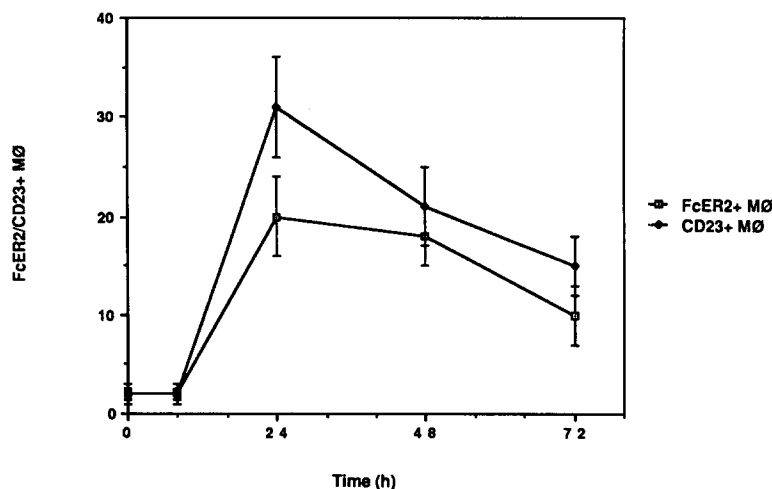


FIGURE 3. Kinetics of the expression of Fc ϵ R2/CD23 induced by rIL-4 on Mo. Purified human Mo were incubated with rIL-4 (50 U/ml) and assessed by flow cytometry at various times for the expression of Fc ϵ R2 (using soluble IgE followed by GAHE-FITC) and CD23 (using mAb 25 followed by GAMIG-FITC). The data represent the mean \pm SE of three experiments.

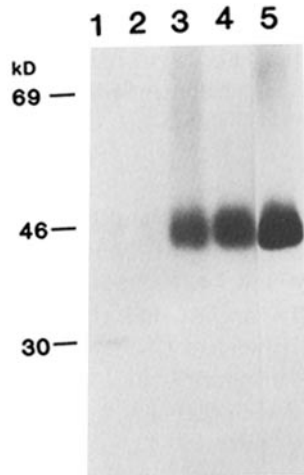


FIGURE 4. SDS-PAGE analysis of FcεR2/CD23 immunoprecipitated from normal human Mo (preincubated with medium or rIL-4), U937 cells, and RPMI 8866 cells. The cells were surface labeled with ^{125}I and lactoperoxidase and then lysed. The cell lysates were then immunoprecipitated with the CD23-specific mAb 25 antibody and analyzed by 12% SDS-PAGE under nonreducing conditions. (Lane 1) Molecular weight markers. (Lane 2) Normal Mo preincubated with medium, (lane 3) normal Mo preincubated with rIL-4 (50 U/ml) for 48 h, (lane 4) U937 cells, (lane 5) RPMI 8866 cells.

ance of a radiolabeled band with an apparent size of 45 kD. This band was almost undetectable on unstimulated Mo (Fig. 4, lane 2) and completely absent in control lanes (data not shown). The 45-kD protein had the same migration pattern under both nonreducing and reducing conditions (data not shown). A 45-kD band could also be precipitated from U937 cells (Fig. 4, lane 4), which constitutively express high amounts of FcεR2/CD23. The bands specifically precipitated by mAb 25 from the lysates of IL-4-induced Mo and U937 cells were identical to the FcεR2/CD23 band precipitated by mAb 25 from RPMI 8866 B cells (Fig. 4, lane 5). The 45-kD protein could be precipitated by IgE as well as by mAb 25 (data not shown). These data further confirm that FcεR2/CD23 can be induced on normal Mo by rIL-4 and that the low-affinity FcεR on Mo and B cells share the CD23 epitope recognized by mAb 25.

rIFN-γ Does not Inhibit IL-4-induced FcεR2/CD23 Expression on Normal Mo. It has recently been reported that the IL-4-dependent induction of FcεR2/CD23 on normal human B cells can be inhibited by rIFN-γ (20, 21). We investigated the effect of rIFN-γ (200 U/ml) on the expression of FcεR2/CD23 induced on

TABLE II
Effects of rIFN-γ on IL-4-induced FcεR2/CD23 and HLA-Dr
Expression by Normal Human Mo

IL	FcεR2 ⁺ /CD23 ⁺	HLA-Dr ⁺ (MCF)
	%	
Nil	7.7 ± 2.5/6.2 ± 2.3	120 ± 7
rIL-4	25.7 ± 4.4/31.0 ± 9.4	129 ± 9
rIFN-γ	7.8 ± 2.5/8.8 ± 3.2	151 ± 2
rIFN-γ + rIL-4	23.6 ± 5.2/28.6 ± 9.4	140 ± 9

Purified human Mo were incubated with rIL-4 (50 U/ml), rIFN-γ (200 U/ml), or rIFN-γ + r-IL4. 48 h later, Mo were assessed by IF for the expression of FcεR2/CD23 and HLA-Dr antigens, as described in Material and Methods. The table shows the mean ± SE of the results obtained in six experiments.

human Mo by rIL-4 (50 U/ml). Although rIFN- γ markedly increased HLA-Dr expression on Mo, it could not inhibit the expression of Fc ϵ R2/CD23 when added to the Mo culture together with rIL-4 (Table II). No effect was observed even when rIFN- γ was added to the culture 36 h before rIL-4 (data not shown).

Discussion

The results of this study show that rIL-4 specifically induces the expression of the low-affinity receptor for the Fc portion of IgE (Fc ϵ R2) on normal human Mo. Fc ϵ R2/CD23 induction could be detected both by flow cytometry (using anti-CD23 mAbs and soluble IgE in one- and two-color IF) and by immunoprecipitation with CD23-specific mAb or IgE coupled to Sepharose. Fc ϵ R2/CD23 on Mo is closely related, if not identical, to the Blast-2 antigen, which has to date been considered as a marker restricted to activated B cells (13, 14). Fc ϵ R2/CD23/Blast-2 is therefore expressed on cells belonging to different lineages.

Fc ϵ R2/CD23 was fully expressed after a 24-h incubation with rIL-4, and was still detectable 72 h after the addition of IL-4. None of the other rILs tested (IL-1, IL-2, IL-3, IL-5, BSF-2, GM-CSF, and IFN- γ) could induce Fc ϵ R2/CD23, either alone or in various combinations. No synergism was observed between IL-4 and other ILs. Under these experimental conditions, human IgE did not significantly increase IL-4-induced CD23 expression on Mo, as assessed by staining with CD23-specific mAbs (Vercelli, D., and D. Y. M. Leung, unpublished observations). PMA \pm ionomycin (data not shown) and IFN- γ , powerful Mo activators able to significantly increase HLA-Dr expression, could not induce Fc ϵ R2/CD23. Such findings suggest that different kinds of stimuli can induce different states of Mo activation. This is consistent with the recent observation that IFN- γ and bacterial surface molecules (such as LPS and muramyl dipeptide) induce different patterns of mRNA expression in Mo (22).

Interestingly enough, the expression of Fc ϵ R2/CD23 on Mo and B cells seems to be regulated differently; IFN- γ was not able to inhibit the IL-4-induced expression of Fc ϵ R2/CD23 on Mo, neither when added to the culture together with IL-4, nor when added 36 h earlier. In contrast, it has been recently reported that IFN- γ has a strong inhibitory effect on the IL-4-dependent induction of Fc ϵ R2/CD23 on human B cells (20). In addition, phorbol esters could not increase Fc ϵ R2/CD23 expression on Mo, while an inducing effect has been recently described for B cells (23, 24). Recent evidence suggests that Fc ϵ R2/CD23 may play a crucial role in growth signal transduction on the B cell surface (23–26). The biological significance of Fc ϵ R2/CD23 on Mo remains to be established.

Previous reports have mainly focused on the various effects of IL-4 on B and T cells (reviewed in Reference 27). However, it has been recently reported that IL-4 is able to activate mouse macrophages, increase their tumoricidal activity and Ia antigens expression (28), and enhance their antigen-presenting capacity (29). The activity of IL-4 on macrophages is consistent with the detection of IL-4-specific high-affinity receptors on murine macrophage lines (30) and thioglycolate-stimulated peritoneal macrophages (31), as well as on the human promonocytic U937 cell line (32). The present study is the first report of an IL-4 effect on normal human Mo. Our results further confirm that IL-4 is a multi-

functional lymphokine, able to act on cells belonging to different lineages.

The observation that IL-4 can strongly induce the expression of FcεR2/CD23 on normal human Mo is of particular interest in view of the role that this lymphokine may have in the regulation of the IgE system. IL-4 has been shown to induce the *in vitro* synthesis of IgE by LPS-activated murine B cells (33). In addition, IL-4 is involved in the *in vivo* control of IgE production; the injection of anti-BSF-1 mAb to mice infected with *Nippostrongylus brasiliensis* has been reported to block the IgE response (34). Finally, we have recently shown that the supernatant of a Th2-like human alloreactive T cell clone, secreting IL-4 and not IFN-γ and IL-2 (35), can induce IgE synthesis by normal B cells, and that this IgE enhancing ability is IL-4 dependent (Jabara H. H., S. J. Ackerman, D. Vercelli, T. Yokota, K. Arai, J. Abrams, A. M. Drorak, M. C. Lavigne, J. Bancheureau, J. de Vries, D.Y.M. Leung, and R. S. Geha, submitted for publication). The same supernatant is also able to induce the expression of FcεR2/CD23 on Mo and B cells (Vercelli D., D. Y. M. Leung, H. H. Jabara, and R. S. Geha, manuscript in preparation). It is therefore likely that IL-4 plays an important, if not crucial, role in the modulation of the IgE system by upregulating not only the synthesis of IgE, but also the expression of its receptors. An increased secretion of IL-4 in allergic disease may be at least partially responsible for the high percentage of circulating Mo bearing FcεR2/CD23 observed in these disorders. The simultaneous presence of a large population of FcεR2/CD23⁺ Mo and high concentrations of serum IgE may enhance receptor-ligand interactions on the Mo membrane and promote the release of Mo-derived mediators important for the inflammatory pathogenesis of allergic reactions.

Summary

rIL-4 (B cell stimulatory factor 1) induces the expression of FcεR2/CD23 on normal human monocytes (Mo). FcεR2/CD23 induction was detectable both by flow cytometry using anti-CD23 mAbs as well as soluble IgE, and by the immunoprecipitation with CD23-specific mAb or IgE of a 45-kD band from ¹²⁵I-lactoperoxidase-labeled Mo. FcεR2/CD23 was fully expressed after a 24-h incubation with rIL-4, and was still detectable after 72 h from the addition of IL-4. This effect was specific, because none of the other rILs tested (IL-1, IL-2, IL-3, IL-5, B cell stimulatory factor 2, granulocyte-macrophage colony stimulating factor, and IFN-γ) could induce FcεR2/CD23, either alone or in various combinations. No synergism was observed between IL-4 and other ILs. IFN-γ was not able to inhibit the IL-4-induced expression of FcεR2/CD23 on Mo, neither when added to the culture together with IL-4, nor when added 36 h earlier.

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