IFN-α and IFN-γ Have Different Regulatory Effects on IL-4-Induced Membrane Expression of FceRIIb and Release of Soluble FceRIIb by Human Monocytes

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We used highly purified human monocytes to study the regulation of cell surface and secretion of the low affinity FcR for IgE (FceRIIb). IL-4 induces FceRIIb expression and soluble FceRIIb release in a dose-dependent manner. Significant levels of FceRIIb expression were obtained after 12 h of incubation with IL-4 and maximal expression was observed between 24 to 48 h after which the expression declined. Surface expression was followed by secretion of soluble FceRIIb which reached maximal levels after 3 to 4 days of incubation and which remained constant throughout 7 days of culture. Induction of FceRIIb expression by IL-4 was completely blocked by anti-IL-4 antibodies. Furthermore, IL-1α, IL-2, IL-5, granulocyte-macrophage-CSF, IFN-α, IFN-γ, low m.w. BCGF and also LPS all failed to induce FceRIIb expression, demonstrating the specificity of the induction. FceRIIb membrane expression induced by IL-4 was reduced in the presence of IFN-γ and IFN-α. Strong inhibition of IL-4-induced FceRIIb expression was observed at IFN-α concentrations of 450 U/ml (80%), and 100 U/ml of IFN-γ reduced IL-4-induced FceRIIb expression by 70%. Interestingly, soluble FceRIIb release was strongly inhibited by IFN-α. In contrast, IFN-γ did not affect soluble FceRIIb release, suggesting that reduced membrane expression of FceRIIb observed in the presence of IFN-γ does not reflect inhibition of FceRIIb expression but may represent enhanced cleavage or reduced anchoring in the membrane of FceRIIb. Finally, IL-5 that has been shown to enhance IL-4-induced FceRIIb on B cells does not enhance significantly IL-4-induced FceRIIb membrane expression or subsequent soluble FceRIIb release by monocytes. Taken together these results show that IFN-α and IFN-γ have different regulatory effects on IL-4-induced FceRIIb membrane expression and soluble FceRIIb release by human monocytes.

Human rIL-4 has pleiotropic effects (1). IL-4 acts as a growth factor for activated T cells, thymocytes, NK cells, and B cells (2, 3). It can enhance specific CTL activity (4), but suppresses IL-2-induced lymphokine-activated killer cell activity (4, 5). IL-4 also induces the expression of the FceRIIb (6), that was found to represent the B cell activation marker CD23 and enhances the expression of class II MHC Ag both on normal and malignant B cells (6-8).

IL-4 promotes the subsequent release of a soluble form of CD23 from B cells (9). Recently B cells were found to express two species of low affinity receptors for IgE FceRIIa and FceRIIb. FceRIIa is constitutively expressed on normal B cells and B cell lines, whereas FceRIIb is inducible by IL-4 on B cells, monocytes, and eosinophils (10).

Whereas IL-4 induces IgE synthesis by normal B cells (11-13), this induction requires both CD4+ T cells and monocytes (11, 14). Binding of IgE to FceRIIb expressed by monocytes and macrophages (15), followed by binding of allergen to these receptors results in macrophage activation (16, 17). Interestingly, induction of FceRIIa and -b expression on human B cells and IgE synthesis by IL-4 was completely blocked by IFN-γ and IFN-α, but induced by IL-5 (7, 11-14). In addition IFN-γ administration in vivo inhibits murine IgE production (18, 19). These results indicate that IFN-γ, IFN-α, and IL-5 together act as regulators of IL-4-induced IgE responses of B cells.

In a previous study (20) we reported that IL-4 enhances the expression of the LFA-1 family molecules CR3 and p150,95 and class II MHC Ag on human monocytes. During these studies we noted that IL-4 also induces expression of FceRIIb. This observation prompted us to investigate the effects of IL-4 and the regulation by IFN-α, IFN-γ, and IL-5 of IL-4-induced FceRIIb expression and subsequent soluble FceRIIb release from human monocytes. In our study it is demonstrated that IL-4 induces a strong induction of FceRIIb that subsequently is released in its soluble form. In addition it is shown that IFN-α and IFN-γ have different regulatory effects on IL-4-induced FceRIIb membrane expression and soluble FceRIIb release, whereas IL-5 was ineffective.

MATERIALS AND METHODS

Isolation of monocytes. Human peripheral blood monocytes were isolated from 500 ml of blood of normal human donors, as described previously (21, 22). The mononuclear cells were separated by density centrifugation with a blood component separator, followed by frac-

Abbreviations used in this paper: FcεRI, low affinity receptor for IgE; BCGF, B cell growth factor; GM-CSF, granulocyte macrophage-CSF; HSA, human serum albumin.
H2O2 Production by Monocytes

There was no difference in the number of cells that were recovered after culture for x days, washed and resuspended in Teflon bags (Jansen MNL, St. Niklaas, Belgium). Monocytes were harvested in a concentration of 4.10^6 cells/ml at 37°C, 5% CO2, and 100% humidity by Teflon bags (Jansen MNL, St. Niklaas, Belgium). Monocytes were harvested after culture for x days, washed and resuspended in PBS containing 0.5% HSA and 0.02% sodium azide and kept on ice until testing. The viability by Trypan blue exclusion exceeded 90%. There was no difference in the number of cells that were recovered after culturing in the presence of different recombiant factors.

Detection of soluble FceRIIb.

The expression of class II MHC Ag on freshly isolated and 1 h adherent monocytes was determined by the binding of the mAb Q5/13 (anti-HLA-DR/DP; IgG2a) (28), provided by Dr. S. Ferrone, Medical College, Valhalla, NY. The expression of FcRRIIb on freshly isolated and cultured monocytes was determined by the binding of the FcRRII/CD23-specific mAb 25 (IgG1) (29). Isotype matched mouse IgG1 and IgG2a antibodies, which did not react with monocytes directed against idiotypic determinants on B cell lymphoma, were used as controls (30).

Immunofluorescence tests were carried out by incubating the monocytes with mAb (in PBS containing 0.5% HSA and 0.02% azide) for 30 min at 0°C. After washing three times with PBS/HSA/azide, the monocytes were incubated with FITC-labeled goat F(ab')2 anti-mouse IgG antibody (Northern Biotech, Minneapolis, MN) for 30 min at 0°C. Subsequently, the cells were washed, suspended in PBS/HSA/azide and analyzed with a FACScan or a FACS IV (Becton Dickinson, Mountain View, CA). Data are expressed as mean fluorescence intensity (arbitrary units) as measured on 4 decades logarithmic amplification (FACScan) and percentage positive cells.

Detection of soluble FcRRIIb.

FcRRIIb in supernatants of monocyte cultures was detected in a sandwich ELISA in which the mAb 25 (IgG1) provided by Dr. S. Ferrone, Medical College, Valhalla, NY has been shown to study the induction of activation markers by IL-4. Freshly isolated monocytes and monocytes cultured in the absence of IL-4 do not express FcRRIIb. However, monocytes express FcRRIIb Ag in a dose-dependent fashion after 40 h of incubation in the presence of IL-4 (Fig. 1A). Significant FcRRIIb expression was already induced at 50 concentrations of 10 U/ml, whereas maximal induction of FcRRIIb expression was observed at 50 to 100 U/ml. Similar induction of soluble FcRRIIb was detected in the supernatant of monocytes cultured with IL-4 (Fig. 1B). IL-4 does not cause a general nonspecific induction of Ag on the cell surface of monocytes because relevant control antibodies, which bind to the monocyte surface, such as leukocyte function-associated Ag 1, are not induced in the presence of IL-4 (20). Kinetics of induction of FcRRIIb expression showed that significant FcRRIIb expression was induced after 12 h of incubation with IL-4, whereas maximal expression was observed between 24 and 48 h of incubation (Fig. 2). Incubation for more than 48 h resulted in a decrease in the expression of FcRRIIb, whereas expression was completely lost at day 5. These data indicate that induction of FcRRIIb expression by IL-4 is transient. This notion was supported by the finding that renewed addition of IL-4 (100 U/ml) on day 4 of the culture had no effect on FcRRIIb expression (results not shown). Furthermore, our data indicate that IL-4 not only induces membrane expression of FcRRIIb, but also results in the subsequent release of relatively large quantities of soluble FcRRIIb (Fig. 2) reaching optimal levels after 3 to 4 days of incubation, which remained unchanged during the next 3 days of culture.

Induction of FcRRIIb expression by IL-4 (100 U/ml) was completely blocked by the anti-IL-4 antisera, demonstrating the specificity of the induction (Fig. 3A). This notion was supported by the finding that neither IL-1α, IL-2, IL-5, GM-CSF, IFN-γ, IFN-α, c-BCGF, nor LPS were able to induce FcRRIIb expression (Fig. 3B), after 20 h of culture. Longer incubation periods (48 to 72 h) also did not induce FcRRIIb expression of cultures negative for FcRRIIb on 20 h (not shown). This indicates that factors known to induce monocyte activation (IL-1α, GM-CSF, IFN-α, IFN-γ, and LPS) are not involved in FcRRIIb expression.

IL-4-induced membrane expression of FcRRIIb is reduced by IFN-γ and IFN-α. We previously demonstrated that IFN-γ and IFN-α can act as antagonists of IL-4. IFN-γ has been shown to block IL-4-induced FcRRIIb expres-
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Methods

Negative control values for all cultures were identical and are represented by the shaded area. The fluorescence intensity of 5000 cells was determined with a FACScan. Soluble FceRIIb present in the culture supernatant was detected with a sandwich ELISA described in Materials and Methods. Values represent one representative experiment of four. SD did not exceed 10% of triplicate determinations.

**Figure 1.** IL-4 induces the expression of FcRIIb (A) and release of soluble FcRIIb (B) from human monocytes. The cells were cultured for 40 h with increasing concentrations of IL-4 as indicated (0, 1, 10, 50, and 100 U). FcRIIb was determined after subsequent staining with the FcRII-specific mAb 25 or mouse IgG1 control mAb and GAM F(ab')2 FITC. Negative control values for all cultures were identical and are represented by the shaded area. The fluorescence intensity of 5000 cells was determined with a FACScan. Soluble FcRIIb present in the culture supernatant was detected with a sandwich ELISA described in Materials and Methods. Values represent one representative experiment of four. SD did not exceed 10% of triplicate determinations.

solution on normal and malignant B cells (7, 8) and both IFN-α and IFN-γ blocked IL-4-induced IgE production by normal B cells (11-14). Fluorescence studies indicated that in the presence of IFN-γ, IL-4 failed to induce membrane expression of FcRIIb on human monocytes (Fig. 4A). The addition of 100 U/ml of IFN-γ resulted in a marked reduction of FcRIIb membrane expression (70%), whereas in the presence of 12.5 U/ml of IFN-γ FcRIIb expression was still significantly lower as compared to control cultures, as judged by both the percentage of FcRIIb+ monocytes and by membrane fluorescence intensity values. Strong inhibitory effects were also observed with IFN-α. IFN-α added at concentrations of 50 U/ml already inhibited membrane FcRIIb expression by more than 50% (Fig. 4B). Eighty percent suppression was observed at 450 U/ml. The IL-4-induced expression of HLA-DR and CR3 was not suppressed by addition of IFN-α or IFN-γ (results not shown). These results indicate that both IFN-α and IFN-γ specifically reduce IL-4-induced FcRIIb membrane expression of human monocytes.

**IL-4-induced release of soluble FcRIIb is blocked by IFN-α but not by IFN-γ.** Although at IFN-γ concentrations of 100 to 200 U/ml no detection of IL-4-induced FcRIIb release was observed, no significant inhibitory effects on IL-4-induced soluble FcRIIb release in the culture medium were shown (Figs. 5B and 6A). In contrast, IFN-α efficiently blocked the release of soluble FcRIIb (Fig. 5A). Concentrations of 50 U/ml already resulted in 50% inhibition, whereas FcRIIb release was blocked for 80% at 450 U/ml of IFN-α. In addition, kinetic studies indicated that IFN-γ tested at 100, 25, and 5 U/ml failed to block IL-4-induced soluble FcRIIb release (Fig. 6A), whereas IL-4-induced FcRIIb membrane expression on day 2 was reduced in a dose-dependent fashion (Fig. 6B). The reduction of FcRIIb membrane expression in the presence of IFN-γ varied from 45 to 71% for six different donors tested (mean 59 ± 12.5). IFN-γ alone did not induce FcRIIb membrane expression (Fig. 3B and 6B) and subsequent soluble FcRIIb release in the culture medium (Fig. 6A). In conclusion, these data indicate that in the presence of IFN-α and IFN-γ a reduced IL-4-induced FcRIIb membrane expression was observed, whereas only IFN-α, and not IFN-γ significantly blocked soluble FcRIIb release from human monocytes.

**IL-5 does not affect IL-4-induced FcRIIb expression and soluble FcRIIb release.** Inasmuch as IL-5 has been shown to enhance IL-4-induced FcRIIb membrane expression and release of soluble FcRII by normal B cells (13), we were interested in the effects of IL-5 on FcRIIb expression and secretion by monocytes. Monocytes were incubated with IL-4 in combination with various concentrations of IL-5. In Figure 7A it is shown that IL-5, which in itself is ineffective (Fig. 3B), did not enhance IL-4-induced FcRIIb surface expression significantly. In addition no effect of IL-5 on IL-4-induced soluble FcRIIb release was observed (Fig. 7B). Taken together, these data indicate that IL-5 has differential effects on IL-4-induced FcRIIb expression on B cells and FcRIIb expression on monocytes.

**DISCUSSION**

Recently we demonstrated that IL-4 induced the expression of class I MHC Ag and CR3 and p150,95 on...
human monocytes (20). These changes in phenotype were accompanied by the induction of macrophage morphology and inhibition of IL-1β and TNF-α synthesis (20, 32). In our study we demonstrate that IL-4 induces the expression of FcεRIIb and subsequent release of soluble FcεRIIb on normal highly purified human monocytes that were not activated by the isolation procedure (23) (Table I). The monocyte preparations were more than 95% pure. Contaminating cells that mainly consisted of granulocytes and lymphocytes were not involved in expression or secretion of FcεRIIb, because addition of various numbers of lymphocytes and granulocytes (up to 10%) did not affect the expression of FcεRIIb by monocytes (results not shown). Induction of FcεRIIb expression on mono-

Figure 3. Specificity of the induction of FcεRIIb by IL-4. A. Induction of FcεRIIb expression is completely blocked by the anti-IL-4 antiserum. The left interrupted line represents the negative control using mouse IgG control mAb. The fluorescence intensity was determined as described in the legend of Figure 1. B. Various other cytokines and LPS do not induce FcεRIIb expression on monocytes after 20 h of culture. FcεRIIb expression was determined as described in the legend of Figure 1. Fluorescence intensity of more than 10,000 cells was determined with the FACS IV. One representative experiment of three is shown.
cyes seems to be an unique property of IL-4, because a large number of cytokines (IL-1α, IL-2, IL-5, GM-CSF, IFN-γ, IFN-α, and low m.w. BCGF) were ineffective despite the fact that many of these factors have been shown to activate monocytes. Furthermore, LPS, which is a strong monocyte activator failed to induce FceRIIb expression. Collectively, these data indicate that IL-4 can specifically activate monocytes to express FceRIIb, because monocytes cultured in the absence of IL-4 remained FceRIIb−.

IL-4 did not only induce the induction of membrane FceRIIb, but also the subsequent release of soluble FceRIIb. Disappearance of FceRIIb at the membrane correlated with the kinetics of soluble FceRIIb release. Soluble FceRIIb levels increased strongly after 72 h of incubation and remained stable from day 4 to 7, indicating that there is no further accumulation of soluble FceRIIb in the supernatants when FceRIIb membrane expression is low or absent. Therefore the disappearance of membrane FceRIIb may reflect release of soluble FceRII. However, a continuous turnover of FceRIIb in the membrane together with a degradation or use of soluble FceRIIb can not be excluded. Finally, the relatively rapid loss of membrane-bound FceRIIb may be due to the inactivation of the gene as has already been suggested by Delespesse et al. (33) and that may be compatible with the observation that renewed addition of IL-4 was ineffective. We demonstrated previously that IL-4 induced FceRII expression on normal and malignant B cells (7, 8) and that this IL-4 effect was blocked by IFN-γ (7, 8, 11-14), but enhanced by IL-5 (13). Here it is shown that both IFN-γ and IFN-α also reduced IL-4-induced FceRIIb membrane expression on monocytes in a dose-dependent fashion. These data are in contrast to those of Vercelli et al. (34), who demonstrated that FceRIIb membrane expression on monocytes induced by IL-4 could not be blocked by IFN-γ. In addition, Delespesse et al. (33) reported that IFN-γ enhanced not only the production of soluble FceRIIb by monocytes and by U937 cells but also induced FceRIIb mRNA expression in these cells. IFN-γ (100 U/ml), which induced class II MHC expression in our experiments (not shown), failed to induce FceRIIb membrane expression. These discrepancies may be related to the different monocyte isolation procedures used by Vercelli et al. (34) and
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Delespesse et al. (33). Adherence of monocytes to plastic surfaces results in activation of these cells and addition of IFN-γ to these activated monocytes may act as a second activation signal resulting in FcεRIIb induction. In addition, other cell types (NK cells) have the capacity to adhere to plastic surfaces (35).

Surprisingly, IFN-γ had no significant inhibitory effects on the secretion of IL-4-induced soluble FcεRIIb, whereas IFN-α that prevented IL-4-induced membrane expression of FcεRIIb more efficiently than IFN-γ, inhibited both surface expression and secretion of soluble FcεRIIb. These results indicate that IFN-α and IFN-γ have different regulatory effects on IL-4-induced FcεRIIb release. Furthermore, if soluble FcεRIIb release is preceded by membrane expression of FcεRIIb, it could be that IFN-γ induces activation of proteases which via an autoproteolytic process immediately cleave the 25-kDa soluble FcεRIIb fragment from the 45-kDa surface FcεRIIb form (33). This implies that we do not deal with inhibition of IL-4-induced membrane expression of FcεRIIb by IFN-γ, but with enhanced autoproteolytic cleavage induced by IFN-γ. Inasmuch as our anti-FcεRII mAb reacts with determinants on the 25-kDa soluble fragment of FcεRII (9), this results in no or strongly reduced levels of FcεRIIb expression on the membrane. Another explanation might be that under the influence of autoproteolytic activity induced by IFN-γ, the truncated 25-kDa FcεRIIb is already cleaved off before the 45-kDa FcεRIIb is anchored in the monocyte membrane. IL-5, which enhanced IL-4-induced FcεRIIb expression, soluble FcεRIIb release and IgE synthesis by normal B cells (13, 14), did not augment IL-4-induced FcεRIIb expression and soluble FcεRIIb release from monocytes. IL-5 was also ineffective when tested alone. Inasmuch as B cells express two species of low affinity receptors for IgE FcεRIIA and FcεRIIB it is presently unclear whether the enhanced FcεRIIb expression observed in the presence of IL-4 plus IL-5 on B cells is related to upregulation of only FcεRIIA expression.

The precise function of FcεRIIb and monocyte-derived soluble FcεRIIb remains to be determined, but soluble FcεRII, purified from supernatants of EBV-transformed
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