IL-4 DECREASES FcγR MEMBRANE EXPRESSION AND FcγR-MEDIATED CYTOTOXIC ACTIVITY OF HUMAN MONOCYTES

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Monocytes can express three classes of FcR for IgG: FcγRI, FcγRII, and FcγRIII (CD64, CD32, and CD16, respectively) of which the FcγRIII is expressed after prolonged culture. FcγR expression is regulated by IFN-γ. Because IFN-γ and IL-4 have antagonistic effects on the expression of the FcR for IgE on human monocytes, we studied the effect of IL-4 on FcγR expression and function. We show that IL-4 down-regulates FcγRI, FcγRII, and FcγRIII expression of cultured monocytes and inhibits IFN-γ enhanced FcγRI expression. Exposure of monocytes to IL-4 for 40 h resulted in a dose-dependent decrease of the expression of all three FcγR that persisted throughout the whole culture period (7 days). Anti-IL-4 antibodies completely reversed the IL-4 effect. In addition the impaired FcγR expression correlated directly with reduced FcγR-mediated function because monocytes cultured in the presence of IL-4 have a reduced capacity to lyse human E opsonized with human IgG anti-D or mouse antilymphocytoma A antibodies. These observations, together with the previous finding that IL-4 induces FcγRIIb expression on monocytes, indicate that IL-4 and IFN-γ may control the FcγR-mediated immune response by differentially regulating FcγR expression.

Soluble mediators influencing monocyte activity include bacteria-derived components, and a number of different lymphokines and monokines (1). IFN-γ is one of the common monocyte activators that induces the expression of class II MHC Ag and of FcR for IgG on human monocytes (2-4). Recently other factors have been described that possess monocyte-activating activity, such as granulocyte-macrophage-CSF (5) and IL-4 (6). Monocytes exposed to IL-4 show enhanced expression of MHC class II Ag, CD11b/CD18, and CD11c/CD18 (6), and display a reduced capacity to secrete IL-1 and TNF-α (7).

IL-4 also induces the expression of the low affinity FcγRII/CD23 (8). IL-4 specifically induces expression of the FcγRIIb, because anti-IL-4 antibodies block IL-4-induced expression and no other lymphokine has a similar effect. This prompted us to investigate the effect of IL-4 on the expression and function of other monocyte FcR. Monocytes can express three classes of FcR for IgG: FcγRI, FcγRII, and FcγRIII (CD64, CD32, and CD16, respectively) (9), of which the FcγRII is only expressed with length of time in culture (10, 11). These FcγR are important mediators in the clearance of immune complexes, phagocytosis, and ADCC activity (12). The results of this study show that IL-4 reduces the expression of all three FcR for IgG on human monocytes that correlate with a reduced capacity to lyse antibody opsonized E.

MATERIALS AND METHODS

Isolation and culture of human monocytes. Human peripheral blood monocytes were isolated from 500 ml of blood of normal human donors, as described previously (13, 14). Mononuclear cells were isolated by density centrifugation in a blood component separator, followed by fractionation into lymphocytes and monocytes by centrifugal elutriation. The monocyte preparation was over 98% pure, as judged by nonspecific esterase staining and contained more than 98% viable cells. Monocytes were cultured in Yssel's medium (15) containing HSA and supplemented with 1% autologous or pooled heat inactivated human serum. This culture medium was endotoxin free as determined by the Limulus ameobocyte lysate assay (less than 0.2 ng/ml of endotoxin). The monocytes were incubated at a concentration of 4 x 10^6 cells per ml in Teflon bags (Jansen MNL, St. Niklaas, Belgium).

After culture for 40 h the monocytes were harvested from the Teflon bags. The cells were washed, resuspended, and kept on ice until use. The viability by Trypan blue exclusion exceeded 90%. There was no difference in the number of cells that were recovered after culture with IL-4 and/or IFN-γ compared with control cultures (over 80%).

rIL-4, rIFN-γ, and anti-IL-4 antiserum. Recombinant human IL-4 was obtained as a purified protein from supernatants of L cells transfected with the cDNA clone encoding IL-4 (5 x 10^9 U/ml), kindly provided by Dr. S. Nagabhushan (Scherings-Plough Corporation, Bloomfield, NJ). One U of IL-4 was defined as the concentration of IL-4 resulting in half maximal proliferation of PHA-activated T lymphoblasts (16). Human rIFN-γ (sp. act. 10^9 U/ml) was kindly provided by Drs. P. Trojta and S. Nagabhushan (Scherings-Plough Corporation). The polyclonal rabbit anti-IL-4 antiserum that blocks the biologic activity of IL-4 as has been described previously (16).

Detection of surface membrane determinants. FcγRI, FcγRII, and FcγRIII expression was measured on monocytes cultured in medium only or in the presence of IL-4 and/or IFN-γ. The anti-FcR antibodies used were 32.2 (IgG2a) and IV.3 (IgG2b) directed against FcγRI and FcγRII, respectively. These mAbs were both purchased from Medarex Inc. (West Lebanon, NH). The anti-FcγRIII used was the CLB/FcR gran 1 (IgG2a) kindly provided by T. W. J. Huizinga (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service).

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*Abbrivations used in this paper: HSA, human serum albumin; ADCC, antibody-dependent cellular cytotoxicity.
**RESULTS**

*IL-4 decreases FcγRI, FcγRII, and FcγRIII surface expression on human monocytes.* Freshly isolated monocytes express at least two FcγR, namely FcγRI and FcγRII, and a third, FcγRIII, is induced after culture. Because IL-4-induced alterations in the expression of surface membrane Ag on human monocytes can in general be detected after culture in 100 U/ml of IL-4 for 1 to 3 days (6), we cultured monocytes for 40 h in the presence or absence of 100 U/ml of IL-4. Figure 1 shows that IL-4 was capable of reducing the membrane expression of FcγRI, FcγRII, and FcγRIII after 40 h of culture. In contrast IL-4 enhanced the expression of HLA-DR/DP Ag and induced expression of FcγRIIb (Fig. 1), indicating that IL-4 does not cause a general nonspecific reduction of Ag expression on the cell surface. Figure 2A shows a time course experiment in which it is demonstrated that 100 U/ml of IL-4 block FcγR expression starting from day 2 of culture and this persisted during the whole culture period (7 days). Figure 2B shows a dose-response curve of the effect of IL-4 on the FcγR membrane expression. The results indicate that maximal decrease in expression of all three FcγR is found at a concentration of 100 U/ml of IL-4. This inhibition of FcγR on monocytes is specific for IL-4 because anti-IL-4 antibodies completely abrogate the IL-4-induced decrease in FcγR membrane expression.

The percentage specific $^{31}$Cr release was calculated according to the formula:

$$\frac{\text{Cr release of sample} - \text{Cr release in medium}}{\text{Cr release in 1% Triton X-100} - \text{Cr release in medium}} \times 100\%$$

The student's $t$-test was used for statistical analysis of the data.
IL-4 reduces FcγR expression and ADCC activity of monocytes.

A. Time course of the effect of IL-4 on the expression of FcγR on human monocytes. The cells were cultured for 0, 1, 2, 3, 5, and 7 days (A) or 40 h (B) with 100 U/ml of IL-4 (A) or increasing concentrations of IL-4 (B). After culture, the fluorescence analysis was carried out as described in the legends of Figure 1. Data are expressed as absolute mean fluorescence intensity (fluorescence index) calculated according to the formula:

\[ \text{FI} = \frac{\text{mFI test sample} - \text{mFI negative control (isotype matched)}}{\text{mFI negative control (isotype matched)}} \]

Figure 3, whereas control rabbit antiserum was ineffective. It seems unlikely that the effects of IL-4 are due to contaminating cells because the monocyte preparations were always more than 95% pure and also because addition of 10% contaminating lymphocytes to pure monocyte fractions did not affect the inhibitory effect of IL-4 on FcγR expression (results not shown).

These results demonstrate that IL-4 induces a reduction of the membrane expression of FcγRI, FcγRII, and FcγRIII on human monocytes but enhances the expression of FcγRIIb.

IL-4 reduces IFN-γ-induced up-regulation of FcγRI on human monocytes. IFN-γ strongly enhances the expression of FcγRI on human monocytes (3, 4). We have previously demonstrated that IFN-γ can act as an antagonist of IL-4 on B cells (22–24) and on monocytes (see footnote 4). Therefore, we studied the effect of IL-4 on the IFN-γ-induced membrane expression of FcγRI. Immunofluorescence studies show that IL-4 inhibits the IFN-γ-induced up-regulation of expression on monocytes (Fig. 4). Maximal inhibition (55%) of FcγRI membrane expression can be achieved using IL-4 concentrations of more than 200 U/ml. A similar type of inhibition can be observed when monocytes are incubated for 24 h with 100 U/ml of IFN-γ before the addition of IL-4. In addition, anti-IL-4 antibodies block the IL-4-induced inhibition showing the specificity of this inhibition (results not shown). Taken together, these data demonstrate that IL-4 partially inhibits the up-regulation of membrane expression of FcγRI in the presence of IFN-γ.

B. Concentration dependent down regulation of FcγR on human monocytes. The cells were cultured for 0, 1, 2, 3, 5, and 7 days (A) or 40 h (B) with 100 U/ml of IL-4 (A) or increasing concentrations of IL-4 (B). After culture, the fluorescence analysis was carried out as described in the legends of Figure 1. Data are expressed as absolute mean fluorescence intensity (fluorescence index) calculated according to the formula:

\[ \text{FI} = \frac{\text{mFI test sample} - \text{mFI negative control (isotype matched)}}{\text{mFI negative control (isotype matched)}} \]

IL-4 reduces ADCC activity of human monocytes. To determine whether reduced FcγR expression reflected reduced functional Fc-induced activity of the cells, we investigated the effect of IL-4 on the capacity to lyse anti-D opsonized human Rh+ E. The observed cytotoxicity toward anti-D opsonized E apparently reflects the activity of FcγRI (11). Figure 5 shows that the ADCC activity of monocytes cultured in the presence of IL-4 is reduced. A similar dose of 100 U/ml of IL-4 that causes maximal reduction in membrane expression of FcγR (Fig. 2) also causes maximal reduction of ADCC. To exclude the possibility that Fc-mediated phagocytosis interferes with ADCC in the 20-h chromium release assay, we carried out short term 4-h chromium release assays in which Fc-mediated phagocytosis does not play a significant role (25). We obtained similar results (not shown) compared
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preferentially bind to FcγRII (8). The results in Figure 6 show that ADCC mediated via murine IgGl can be significantly reduced when the effector monocytes are cultured in the presence of IL-4. To demonstrate that the reduced ADCC activity of monocytes cultured in the presence of IL-4 is specific, we cultured monocytes with and without IL-4 in the presence of anti-IL-4 antiserum. The results in Figure 7 show that anti-IL-4 antiserum completely neutralized the inhibitory effects of IL-4, whereas control rabbit antiserum was ineffective.

Taken together the results demonstrate that IL-4-reduced membrane expression of FcγR correlates well with the reduced capacity to lyse antibody-coated targets.

IL-4 decreases IFN-γ-induced ADCC activity of human monocytes. IFN-γ not only stimulates the expression of the FcγRI on monocyte membranes but also enhances ADCC activity mediated via this receptor (26). Inasmuch as IL-4 reduces the IFN-γ-induced membrane

Figure 4. IL-4 inhibits IFN-γ-enhanced FcγRI expression of monocytes. The cells were cultured for 40 h with and without 100 U/ml of IFN-γ and increasing concentrations of IL-4. Fluorescence analysis was carried out as described in the legends of Figure 1. One representative experiment of three is shown.

Figure 5. IL-4 inhibits ADCC activity of monocytes against anti-D-coated human E. The monocytes were cultured for 40 h in increasing concentrations of IL-4. ADCC tests were performed as described in Materials and Methods. Data are expressed as percentage 51Cr release. SD did not exceed 10%. One representative experiment of six is shown.

Figure 6. ADCC activity of monocytes cultured for 40 h in increasing concentrations of IL-4 against anti-D-coated and mouse anti-glycophorin A mAb-coated human E. ADCC tests were performed as described in Materials and Methods. Data are expressed as percentage 51Cr release. E/T ratio was 0.3. SD did not exceed 10%. One representative experiment of three is shown.

Figure 7. Addition of anti-IL-4 antiserum to human monocyte cultures in the presence of 100 U/ml of IL-4 completely restores ADCC activity against anti-D-coated human E. The cells were cultured for 40 h with or without 100 U/ml of IL-4 and increasing concentrations of anti-IL-4 antiserum. ADCC tests were performed as described in Materials and Methods. Control rabbit antiserum was ineffective (results not shown). Data are expressed as percentage 51Cr release. E/T ratio was 0.4. SD did not exceed 10%. One representative experiment of three is shown.
expression of FcγRI on monocytes, we tested the ADCC activity of monocytes cultured with IFN-γ in the presence of increasing concentrations of IL-4. The results presented in Figure 8 show a dose-dependent decrease in ADCC activity of IFN-γ-stimulated monocytes in the presence of IL-4. These data indicate that not only the membrane expression of FcγRI is reduced on IFN-γ-stimulated monocytes upon culture with IL-4, but also their capacity to lyse antibody-coated targets.

DISCUSSION

The expression of FcγR on human monocytes is readily regulated by endogenous stimuli of which IFN-γ is the most important. We present data showing that IL-4 causes a decrease in expression of FcγR by human monocytes, which directly correlates with FcγR-mediated function as measured by ADCC activity. The finding that IL-4 is a lymphokine that is capable of affecting FcγR expression is in accordance with the observation that IL-4 induces a loss of FcγRII ligand binding activity on B lymphocytes (27) and that IL-4 inhibits the enhanced expression of FcγRI on B cells (28). This indicates that IL-4 is not only involved in the regulation of FcγR-mediated function of monocytes but also of B lymphocytes.

Because in previous studies time course experiments indicate that changes in surface membrane expression of human monocytes could be detected after 2 days of culture (6), the experiments shown here were carried out with monocytes cultured for 40 h (Fig. 2A). After 2 days of culture not only IL-4-induced MHC class II Ag expression, CD11b/CD18 and CD11c/CD18 expression (6), but also FcγRI expression can be observed on human monocytes (8). A similar time course of FcγRI, IL-2R and MHC class II Ag expression was found after IFN-γ treatment of monocytes (29). In addition after 2 days of culture human monocytes express not only FcγRI and FcγRII but also FcγRIII. The individual contribution of the FcγR to ligand binding and functional activity are not yet clearly identified although it has been suggested that the ADCC against E opsonized with human anti-D antibodies is mediated via FcγRI because blockade of FcγRI abrogated this ADCC (11). Furthermore, enhanced FcγRI expression observed in the presence of IFN-γ (Fig. 4) results in strong ADCC activity against anti-D opsonized E (Fig. 8). Blockade of FcγRII abrogated the ADCC activity against E opsonized with IgG murine antibodies (30). We exploited the preferential binding of human IgG to FcγRI and mlgG1 to FcγRII to elucidate the relative contribution of the FcγRI and FcγRII in FcγR-mediated cytotoxic activity. The results demonstrate that the IL-4-induced reduction in membrane expression of FcγRI and FcγRII correlates well with a reduced capacity to lyse human polyclonal IgG anti-D or mlgG1 antglycophorin A opsonized human E. Especially the IFN-γ-induced FcγRI expression and cytotoxic function is down regulated by IL-4 (Figs. 4 and 8). FcγRIIab is predominantly expressed by cultured monocytes and although it is capable of binding ligand (10, 11), it has recently been reported that the newly expressed FcγRIIab on monocytes is not involved in the induction of ADCC of human E opsonized with either anti-D or antglycophorin A (11). Although the reduced FcγR expression correlates well with a reduced ADCC activity we cannot exclude the possibility that there are other monocyte Ag and/or functions important for mediating ADCC activity down-regulated in the presence of IL-4. An indication that IL-4 not only inhibits ADCC by decreasing FcγR expression, but also affects the O2- metabolism is shown by Lehn et al. (31) who describe that IL-4 inhibits the IFN-γ-Induced H2O2 production of human cultured monocytes.

In addition to IFN-γ and IL-4, other factors have been shown to regulate FcγR expression of one or more FcγR. LPS has been described to inhibit IFN-γ-induced FcγR expression on human monocytes and this effect is thought to be mediated by IL-1 and TNF produced by monocytes after exposure to LPS (32). It is very unlikely that IL-1 and TNF are responsible for the inhibitory effect of IL-4 on FcγR expression on human monocytes shown in this study, because IL-4 strongly blocks secretion of IL-1 and TNF by these cells (7) (see footnote 3). However, we cannot exclude the possibility that other IL-4 induced factor(s) are responsible for the down-regulation of FcγR.

A number of studies have shown that IFN-γ has antagonistic effects on IL-4-induced expression of FcγRII and class II MHC Ag on normal and malignant B cells (22-24). IFN-γ also blocks IL-4-induced FcγRIIb expression on human monocytes (see footnote 4). We show that IL-4 has antagonizing effects on IFN-γ-induced FcγR expression and FcγR-mediated cytotoxic function. These results suggest that IFN-γ is a lymphokine specifically involved in FcγR regulation and IgG responses by stimulating FcγR expression and function and reducing FcγR expression, whereas IL-4 is a lymphokine specifically involved in FcγR regulation and IgE responses because it induces FcγR expression and inhibits FcγR expression and function. Fine-regulation of the individual production of IFN-γ and IL-4 may therefore control the FcR surface expression and functional activity of the FcR, thus regulating the direction of the FcR-mediated immune response.

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