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IL-10 STIMULATES MONOCYTE FcγR SURFACE EXPRESSION AND CYTOTOXIC ACTIVITY

Distinct Regulation of Antibody-Dependent Cellular Cytotoxicity by IFN-γ, IL-4, and IL-10[1]

ANJE A. TE VELDE,[2*] RENE de WAAL MALEFIJT,[†] RICHARD J. F. HUIJBENS,[*] JAN E. de VRIES,[†] AND CARL G. FIGDOR[3*]

*From the *Division of Immunology, The Netherlands Cancer Institute, Antoni van Leeuwenhoek Huis 1066 CX Amsterdam, The Netherlands; and [†]DNAX Research Institute of Molecular and Cellular Biology Inc., Palo Alto, CA 94304*

T cell-derived cytokines IFN-γ and IL-4 have different regulatory effects on two functionally important molecules on human monocytes: MHC class II Ag and the Fc receptor for monomeric IgG, FcγRI (CD64). MHC class II Ag, and FcγRI are both upregulated in the presence of IFN-γ. IL-4 induces MHC class II Ag expression but reduces FcγRI expression. Recently, we showed that the cytokine IL-10 also affects MHC class II Ag expression. Here, we demonstrate that in contrast to the down-regulation of MHC class II Ag expression, IL-10 stimulates FcγRI expression on human monocytes comparable to the levels of FcγRI expression induced by IFN-γ. The IL-10-induced FcγRI expression is specific because anti-IL-10 antibodies completely reverse the IL-10-induced surface expression of FcγRI and correlate with an enhanced capacity to lyse anti-D-coated human rhesus-positive erythrocytes. IL-10 fails to induce the expression of FcγRII (CD32) and FcγRIII (CD16). Furthermore, we demonstrate that IL-10 is able to prevent down-regulation in surface membrane expression of all three FcγR that can be found when monocytes are cultured in the presence of IL-4. In contrast to IFN-γ, IL-10 does not restore the reduced antibody-dependent cellular cytotoxicity (ADCC) activity of IL-4-cultured monocytes. Together, these results show that, similar to IFN-γ, IL-10 is capable of enhancing FcγR expression and ADCC activity, and that IFN-γ, IL-4, and IL-10 have different regulatory effects on both monocyte Ag-presenting capacity and ADCC activity.

IL-10 has been shown to have important pleiotropic biologic effects both in mouse and in man. Murine IL-10, produced by Th2 cells, inhibits the synthesis of cytokines by Th1 (1) and acts as a growth stimulatory factor together with IL-2 and IL-4 on mature and immature T cells (2). In addition, mIL-10 is capable of stimulating growth of mast cells if added together with IL-3 and/or IL-4 (3). Furthermore, it has been demonstrated that mouse B cells show increased expression of class II MHC Ag and remain viable for a prolonged period of time when they are cultured with IL-10 (4). Unlike mouse IL-10, human IL-10 is produced by T cells, B cells, and monocytes (4, 5, J. Abrams et al., manuscript in preparation). Human IL-10 has cytokine synthesis inhibition factor activity on T cells and on monocytes (6) similar to what has been found with mouse IL-10. Apart from the observed reduction in monokine production by monocytes, IL-10 has a strong down-regulatory effect on constitutive and IFN-γ and IL-4-induced MHC class II Ag expression, which results in a reduced capacity of these cells to present Ag (7). These observations suggest that IL-10 in an autocrine fashion is a negative regulator of the initiation of immune responses. An other important function of monocytes is the Fc receptor-mediated effector function. Fc receptors for IgG on human peripheral blood monocytes are involved in many activities of these cells, including clearance of immune complexes, phagocytosis, and ADCC[4] (8). Three classes of FcγR can be distinguished on human monocytes: FcγRI (CD64), FcγRII (CD32), and the FcγRIII (CD16) (9), which is only expressed after prolonged culture (10, 11). The FcγRI membrane expression on human monocytes can be regulated by different cytokines (12–14). IFN-γ induces FcγRI expression (5- to 10-fold) and FcγRI-mediated cytotoxic activity (12, 13), whereas we showed that IL-4 down-regulates expression of all three FcγR and FcγR-mediated cytotoxic activity (14).

In the present study, we have assessed the effect of IL-10 on FcγR surface expression and FcγR-mediated cytotoxic activity, and we demonstrate that IL-10 stimulates monocyte-mediated ADCC.

MATERIALS AND METHODS

Monocyte isolation and culture. Isolation and culture of highly purified human peripheral blood monocytes were performed as described previously (15). Briefly, 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 95% pure, as judged by

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[1] This work was supported by a grant from the Dutch Cancer Society (Project number NKI 86-01).
[2] Present address: Laboratory of Experimental Internal Medicine, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.
[3] Address all correspondence and reprint requests to C. G. Figdor, Division of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.
[4] The abbreviation used is ADCC, antibody-dependent cellular toxicity.

nonspecific esterase staining and contained more than 98% viable cells. Monocytes were cultured in Yssel's medium (16) containing human serum albumin and supplemented with 1% autologous or pooled heat-inactivated human serum. This culture medium was endotoxin free as determined by the Limulus amebocyte lysate assay (less than 0.2 ng/ml of endotoxin). The monocytes were incubated at a concentration of 4×10^6 cells/ml in Teflon bags (Jansen MNL, St. Niklaas, Belgium).

After culture for 40 h, monocytes were collected for determination of FcR expression and ADCC activity. The viability of the cells determined by trypan blue exclusion always exceeded 90%.

Reagents. rIL-4 and IFN-γ were obtained as described previously (14). rIL-10 were expressed in *Escherichia coli* as glutathione-S-transferase fusion proteins, purified and digested with thrombin to remove the *N*-terminal fusion part, yielding active human IL-10 (17). The neutralizing mAb 19F1 (IgG2a) recognizing hIL-10 has been described (J. Abrams manuscript in preparation).

The source for mAb 32.2 (anti-FcγRI, IgG2a) and mAb IV.3 (anti-FcγRII, IgG2b) was Medarex Inc. (West Lebanon, NH). The anti-FcγRIII mAb was the CLB/FcR gran 1 (IgG2a) kindly provided by T. W. J. Huizinga of the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands. MHC class-II Ag were detected using Q5/13 (IgG2a) that detects a determinant common to HLA-DR and HLA-DP molecules (18). Q5/13 was a gift from Dr. S. Ferrone, Medical College, Valhalla, NY.

Facs analysis. Fluorescence analysis of FcγRI, FcγRII, and FcγRIII membrane expression was performed as described previously (14). Before the immunofluorescence test, the monocytes were incubated in human serum in PBS for 30 min to avoid nonspecific binding of the mouse IgG2a Fc region of the test mAb to the FcγRI.

ADCC. ADCC activity of cultured human monocytes against antibody-coated rhesus-positive human erythrocytes was performed as described previously (14).

The Student's *t*-test was used for statistical analysis of the data.

RESULTS AND DISCUSSION

IL-10 induces FcγRI, but not FcγRII and FcγRIII membrane expression on human monocytes. Freshly isolated monocytes express at least two FcγR, namely FcγRI and FcγRII, and a third, FcγRIII, is induced after culture for 40 h. The expression of FcγRI on human monocytes is positively regulated by IFN-γ (12, 13) and down-regulated in the presence of IL-4 (14). Alterations in expression induced by these cytokines generally can be detected after culture for 1 to 3 days (14). In order to determine the effects of IL-10 on FcγR expression, we cultured monocytes in the presence of IL-10 for 40 h and compared the expression of the FcγRI with the expression of monocytes cultured in IL-4 or IFN-γ for a similar period. Figure 1*D* shows that IL-10 (100 U/ml) significantly enhances cell surface expression of the FcγRI as compared to control cultures in medium alone (Fig. 1*A*). Furthermore, and in accordance with previous findings, it is demonstrated that the surface expression of the FcγRI is enhanced or reduced when monocytes are cultured in the presence of IFN-γ or IL-4, respectively (Fig. 1 *B* and *C*) (12–14). In Figure 1 *E* to *H*, the effect of these three cytokines on the expression of MHC class II Ag demonstrates that these monocytes react with the cytokines as described before (7, 15). The dose-response curve in Figure 2 indicates that maximal surface expression of the FcγRI is obtained at concentrations of 300 U/ml of IL-10. This enhanced expression of the FcγRI is specific for IL-10, because the anti-IL-10 mAb 19F1 completely abrogated the IL-10-induced increase in FcγRI membrane expression (Fig. 3).

IL-10 and IFN-γ both induce FcγRI expression on human monocytes and combination of these two cytokines has no additive effects on the FcγRI membrane expression (Table I).

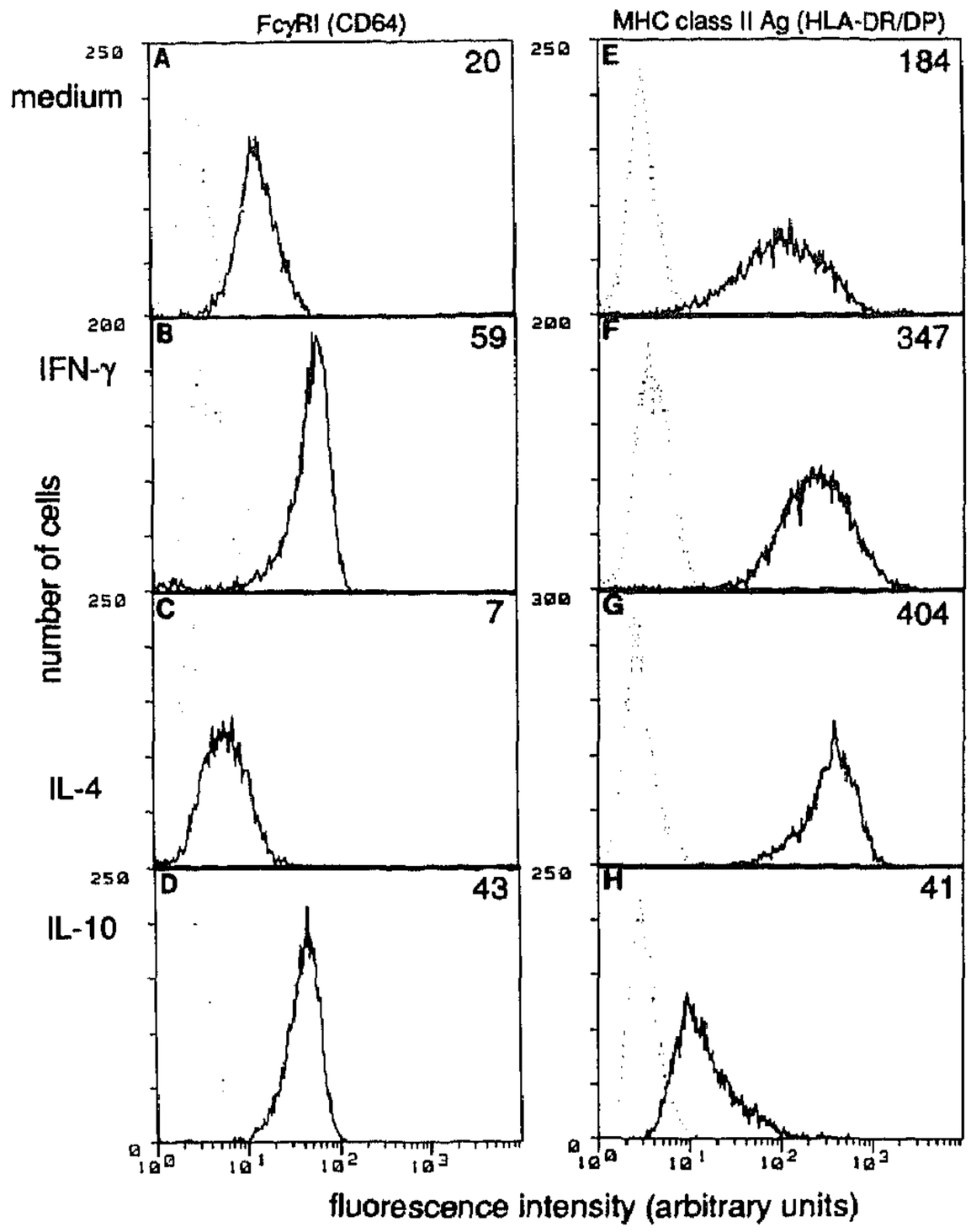

Figure 1. Effect of IFN-γ, IL-4, and IL-10 on monocyte membrane expression of FcγRI (CD64) and MHC class II Ag (HLA-DR/DP). The cells were cultured for 40 h with and without 100 U/ml of IFN-γ, IL-4, or IL-10. FcγRI (*A–D*) and MHC class II Ag (*E–H*) were detected after subsequent staining with specific mAb and GAM F(ab)$_2$ FITC. The fluorescence intensity of 5000 cells was determined with a FACScan. ....., represent the negative controls. The mean fluorescence intensity values of the positive samples are given in the upper right corner. One representative experiment of eight is shown.

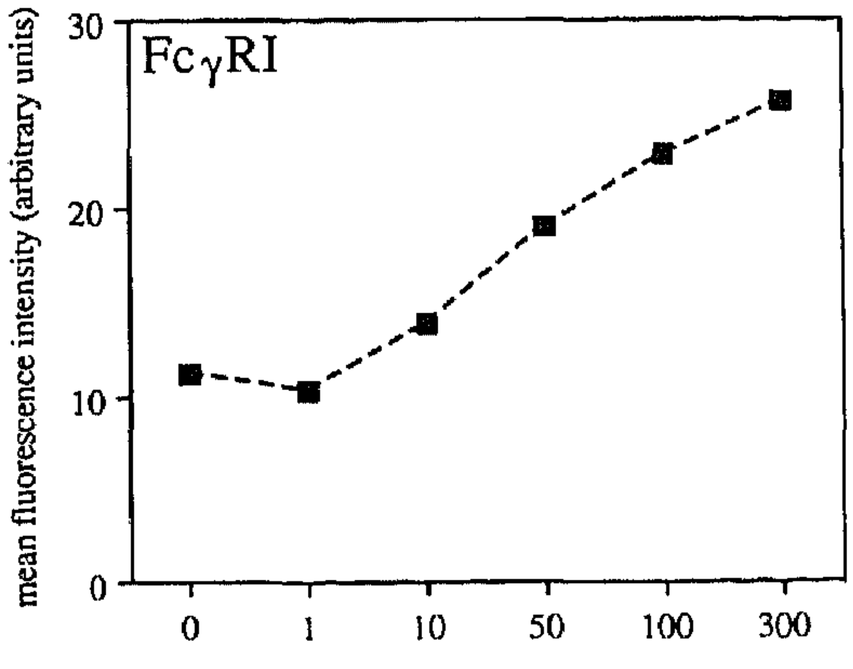

Figure 2. Dose-response curve of the induction of the FcγRI on human monocytes by IL-10. The cells were cultured for 40 h with increasing concentrations of IL-10. Fluorescence analysis was carried out as described in the legend of Figure 1. One representative experiment of three is shown.

We have demonstrated previously that IL-4 down-regulates the membrane expression of the FcγRI, FcγRII, and FcγRIII on human monocytes (14). IL-4-induced down-regulation of FcγRI could be neutralized by IFN-γ (14). The data in Figure 4*B* demonstrate that IL-10 also neutralizes IL-4 down-regulated expression of FcγRI to medium values, similar to IFN-γ (Table I). IL-10 only

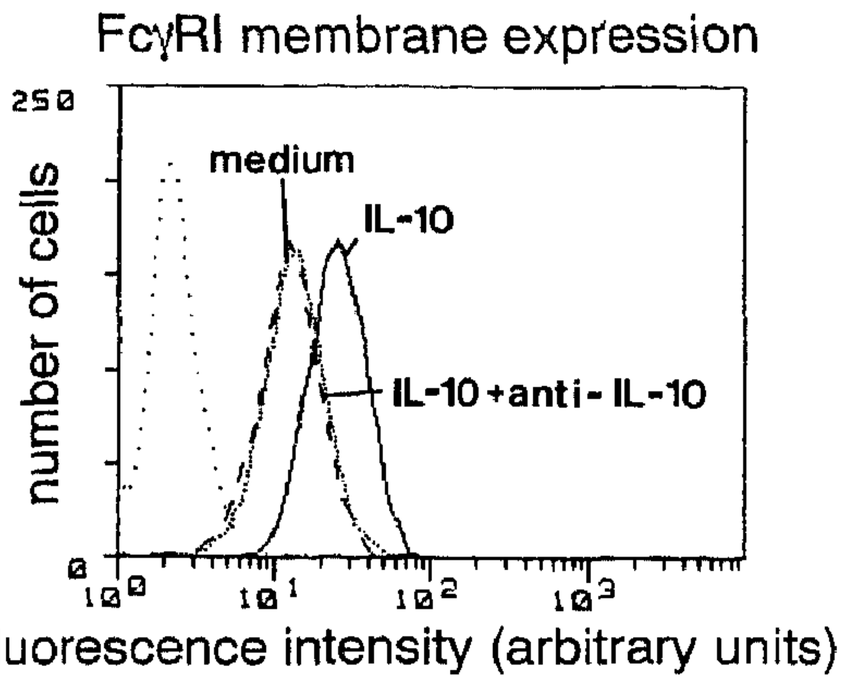

Figure 3. Addition of mAb against IL-10 to human monocytes cultured in the presence of 100 U/ml of IL-10 completely restores FcγRI expression to medium values. Control mAb (IgG2a) was ineffective (results not shown). Fluorescence analysis was carried out as described in the legend of Figure 1. One representative experiment of three is shown.

TABLE I

Effect of IL-4, IL-10, and IFN-γ on monocyte membrane expression of FcγRI[a]

Culture Condition	Percentage of FcγRI Surface Expression Compared to Medium Values	
	No IL-10	With IL-10
Medium	100[b]	175 ± 19[c]
IL-4	55 ± 21[c]	159 ± 48[d]
IFN-γ	244 ± 59[c]	248 ± 41[c]
IL-4 + IFN-γ	125 ± 29	138 ± 19

[a] The monocytes were cultured for 40 h with medium only or 100 U/ml of IL-4, IL-10, and IFN-γ as indicated. The fluorescence analysis was carried out as described in the legends of Figure 1.

[b] The mean fluorescence intensity of the medium cultures was 17, 28, 20, and 13 for cultures from four different donors.

[c] $p < 0.0025$ (compared to medium only).

[d] $p < 0.025$ (compared to medium only).

enhances the expression of FcγRI, and has no effect on the membrane expression of FcγRII and FcγRIII. However, IL-10 neutralized the down-regulatory effects of IL-4 on FcγRII and FcγRIII expression (Fig. 4 *C* and *E*). The level of expression of all three FcγR on monocytes cultured in the presence of combinations of IL-4 and IL-10 were significantly higher than the expression of those in medium alone (Fig. 5 *B*, *D*, and *F*).

ADCC activity of monocytes cultured in the presence of IL-10. The level of ADCC activity of human monocytes has been shown to correlate with FcγR expression (19). Therefore, we tested whether enhanced FcγRI expression induced by IL-10 correlated with an enhanced ADCC activity against anti-rhesus D opsonized human E. Figure 5*A* shows that IL-10 enhances this ADCC activity in a dose-dependent manner. A 2-fold increase in ADCC activity was observed at an IL-10 concentration of 300 U/ml. Similar results were obtained with IFN-γ (Fig. 5*B*). It has been shown that IFN-γ and IL-4 have antagonistic activating effects on human monocytes (14, 20–22). We previously demonstrated that the IFN-γ-induced ADCC activity of human monocytes can be reduced in the presence of IL-4 (14). Therefore, we tested if the enhanced ADCC activity of human monocytes cultured with IL-10 is also down-regulated in the presence of IL-4. Figure 6 shows that not only IFN-γ, but also IL-10-induced ADCC activity is reduced in the presence of IL-4. The data presented in this Figure demonstrate that the IL-4 effect on ADCC activity of IL-10-stimulated monocytes is stronger than the IL-4 effect on ADCC activity of IFN-γ-

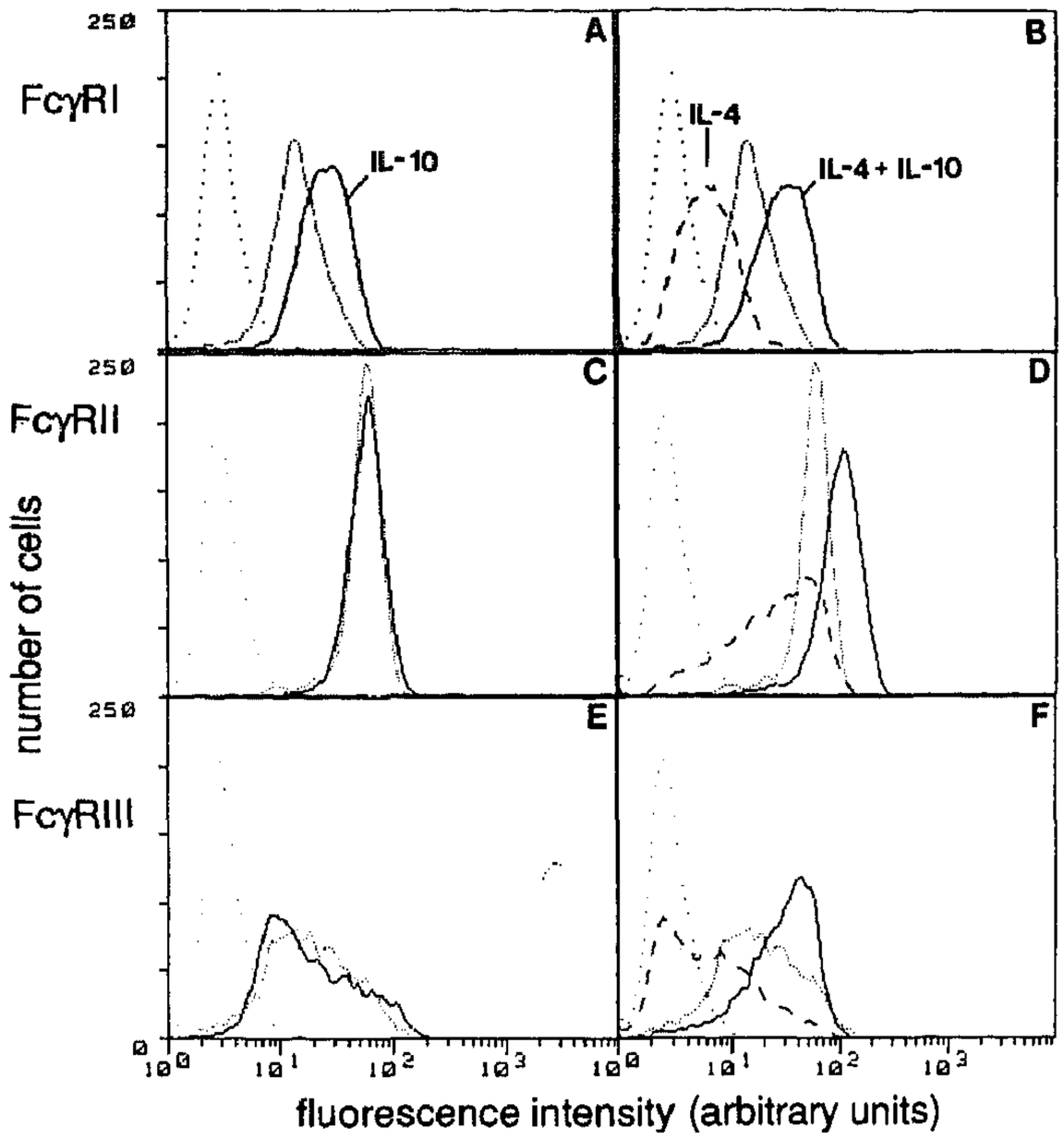

Figure 4. Reversal of IL-4-induced down-regulation of FcγRI, FcγRII and FcγRIII membrane expression by IL-10. The cells were cultured for 40 h. Fluorescence analysis was carried out as described in the legend of Figure 1. In *A* to *F* ...., negative control values; and ----, values cultured in medium alone; ————, in *A*, *C*, and *E* = 100 U/ml of IL-10, and in *B*, *D*, and *F* = IL-10 + 100 U/ml of IL-4. (– – –), IL-4 alone. One representative experiment of three is shown.

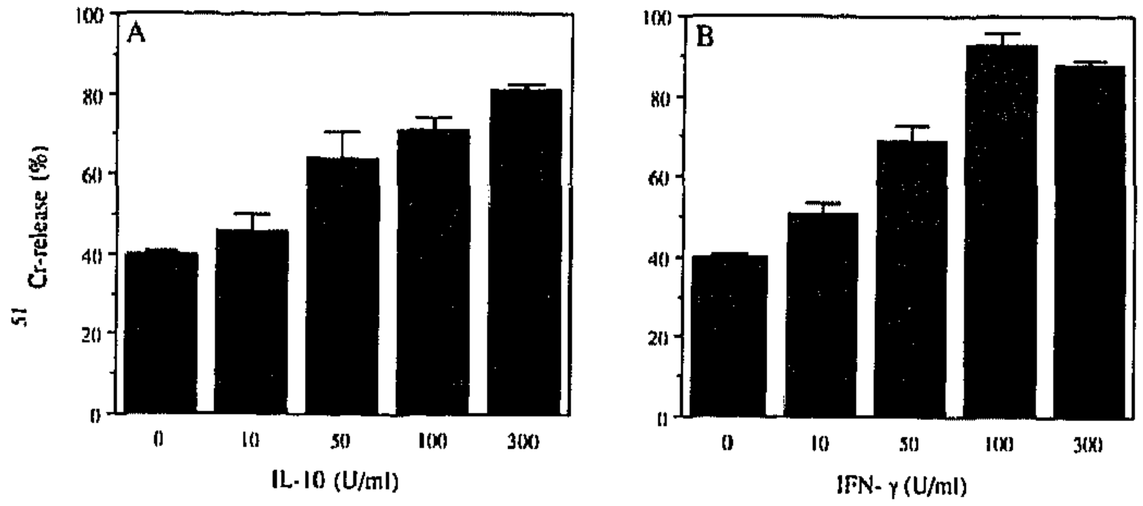

Figure 5. ADCC activity of monocytes cultured for 40 h in increasing concentrations of IL-10 (*A*) compared to IFN-γ (*B*) against anti-D-coated human E. ADCC tests were performed as described in *Materials and Methods*. Data are expressed as percentage of ^{51}Cr release. E/T ratio was 0.6. One representative experiment of three is shown.

activated monocytes.

The ADCC activity of monocytes cultured for 1 to 2 days in medium against anti-rhesus D opsonized human erythrocytes is generally decreased compared to the ADCC activity of freshly isolated monocytes (14, 19). Culture in the presence of IFN-γ restores the ADCC activity to the values obtained when freshly isolated monocytes were used. A possible regulatory mechanism is the modulation of a receptor that mediates this ADCC activity, the FcγRI. The results described in this study suggest that not only IFN-γ, but also IL-10 is a cytokine that regulates monocyte FcγRI membrane expression and ADCC activity.

The observed discrepancy between IFN-γ and IL-10 in counteracting the effect of IL-4 on ADCC activity could not be explained by differences in up-regulation of IL-4 down-regulated expression of FcγRI. Both IFN-γ and IL-10 induce IL-4 down-regulated expression of FcγRI (Table

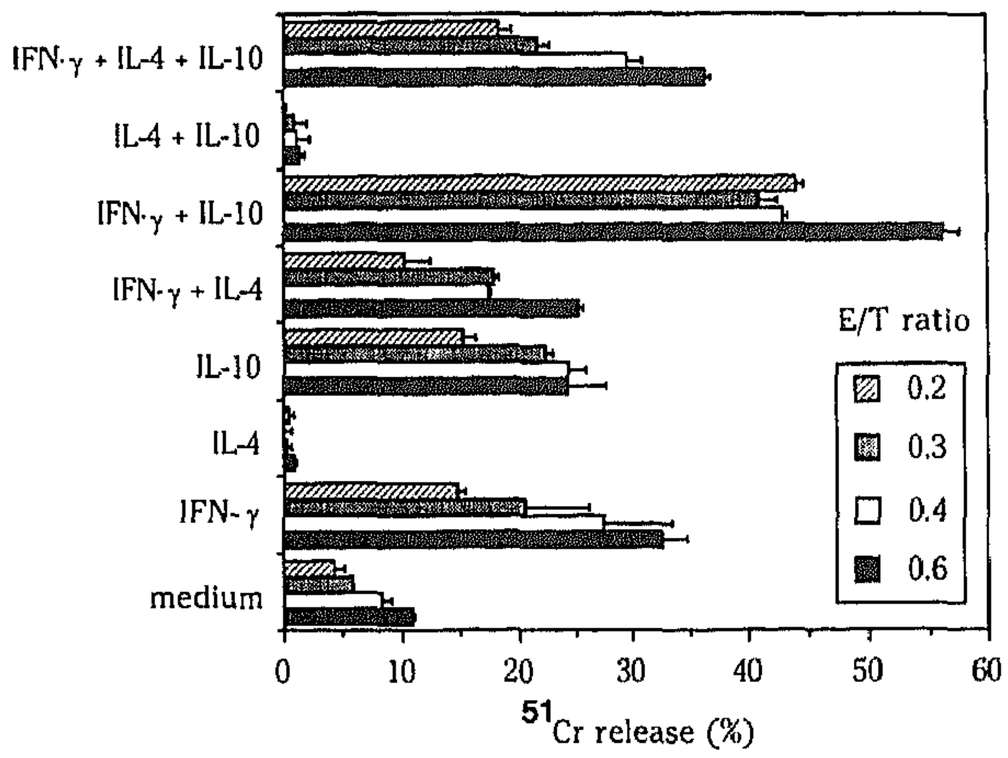

Figure 6. ADCC activity of monocytes cultured for 40 h in combinations of 100 U/ml of IL-4, IFN-γ, and IL-10. ADCC tests were performed as described in *Materials and Methods*. Data are expressed as percentage of ^{51}Cr release. One representative experiment of three is shown.

TABLE II

Effect of IL-4, IL-10, and IFN-γ on class II MHC Ag and FcγRI membrane expression and cytokine synthesis by human monocytes

Monocyte Property	IL-4	IL-10	IFN-γ
Class II MHC Ag	↕	↓	↑
FcγRI	↓	↑	↑
Production of cytokines	↓	↓	↑

I and Fig. 4). Although a close correlation between ADCC activity and level of surface expression of FcγRI is described (14, 19), other monocyte Ag and/or functions are probably important in mediating ADCC activity. One of these functions is the production of reactive oxygen or nitrogen intermediates. Modulation by cytokines of the O_2-metabolism could change the ADCC activity of monocytes. Lehn et al. (22) have described that IL-4 inhibits the IFN-γ-induced H_2O_2 production of human cultured monocytes. Recently, Bogdan et al. (23) demonstrated that IL-10 reduces the H_2O_2 production by mouse peritoneal macrophages. The production of reactive nitrogen intermediates is also inhibited by IL-10 in a murine macrophage cell line (24). A possible reduction of these reactive oxygen and nitrogen intermediates by IL-10 in the presence of IL-4 could explain the difference in the modulation of IL-4 down-regulated ADCC activity by IL-10 and IFN-γ. Because cytokines have been shown to have different as well as comparable effects on monocytes/macrophages of human and mouse origin, conclusions concerning interspecies monocyte/macrophage responsiveness must be drawn with caution.

Also the production of potential cytotoxic cytokines is inversely regulated by IL-4 and IFN-γ (21, 25). IFN-γ has been shown to stimulate cytokine synthesis by human monocytes (21). IL-10, originally described as a cytokine synthesis inhibition factor, strongly inhibits the secretion of IL-1α, IL-1β, IL-6, IL-8, and TNF-α by human monocytes (7). Also different from IFN-γ, IL-10 strongly down-regulates the constitutive class II MHC Ag expression on human monocytes (6). IL-10 not only reduces constitutive class II MHC Ag expression, but also blocked the class II MHC-enhancing effects of IFN-γ and IL-4 in a dose-dependent fashion (6). The effects of IL-4, IL-10, and IFN-γ on membrane expression of class II MHC Ag expression and FcγRI expression and cytokine production by monocytes are summarized in Table II. The actual intensity of the membrane expression of these two molecules and the functional activity of the monocytes depends on the presence of these individual cytokines.

Based on its properties described thus far, IL-10 is shown to be a potent suppressor of immune and inflammatory responses (6, 7). This paper demonstrates that IL-10 can not be considered a general inhibitor of immune responses, since it can stimulate ADCC activity.

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