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Analysis of the Multiple Interactions Between IL-12 and the High Affinity IL-12 Receptor Complex

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IL-12 is a heterodimeric cytokine, composed of a p40 and a p35 subunit, that exerts its biological effects by binding to specific cell surface receptors. Two IL-12R proteins, designated human IL-12 (huIL-12) receptor β1 (huIL-12Rβ1) and huIL-12Rβ2, have been previously identified. These IL-12R individually bind huIL-12 with low affinity and in combination bind huIL-12 with high affinity and confer IL-12 responsiveness. In this study the interactions of huIL-12 with these two identified human IL-12R protein subunits are examined. The heterodimer-specific anti-huIL-12 mAb 20C2, which neutralizes huIL-12 bioactivity but does not block ^{125}I-huIL-12 binding to huIL-12Rβ1, blocked binding of huIL-12 to huIL-12Rβ2. In contrast, anti-huIL-12Rβ1 mAb 2B10 and mouse IL-12 p40 subunit homodimer (mo(p40)$_2$) blocked ^{125}I-huIL-12 binding to huIL-12Rβ1, but not to huIL-12Rβ2. Therefore, two classes of IL-12 inhibitors can be identified that differ in their ability to block huIL-12 interaction with either huIL-12Rβ1 or huIL-12Rβ2. Both mo(p40)$_2$ and 20C2 blocked high affinity binding to huIL-12Rβ1/β2-cotransfected COS-7 cells, although, as previously reported, mo(p40)$_2$ does not block high affinity binding to IL-12R on PHA-activated human lymphoblasts. Furthermore, these two classes of IL-12 inhibitors synergistically decreased huIL-12-stimulated proliferation and IFN-γ production. Therefore, IL-12, in binding to the high affinity IL-12R, interacts with IL-12Rβ1 primarily via regions on the IL-12 p40 subunit and with IL-12Rβ2 via 20C2-reactive, heterodimer-specific regions of IL-12 to which the p35 and p40 subunits both contribute. ***The Journal of Immunology,*** **1998, 160: 2174–2179.**

IL-12 is an immunomodulatory cytokine produced primarily by APCs that plays an important role in promoting Th1-type immune responses and cell-mediated immunity (1–6). Among its regulatory activities, IL-12 stimulates the proliferation of activated T and NK cells (7–10), enhances the lytic activity of lymphokine-activated killer (LAK)/NK cells and CTL (7, 9, 11), and induces the production of IFN-γ by both T and NK cells (7–9, 12). IL-12 mediates its biologic activities through binding to specific cell surface receptors. This IL-12R was initially characterized on PHA-activated lymphoblasts and IL-2-activated NK cells (13, 14). These cells display at least two classes of sites that bind ^{125}I-labeled human IL-12 (^{125}I-huIL-12)[2] with K_d values of 5 to 20 pM and 2 to 6 nM (15, 16). The IL-12R has recently been shown to be composed of at least two protein subunits, IL-12Rβ1 and IL-12Rβ2 (15, 17, 18). Each of the identified IL-12R subunits is a member of the cytokine receptor superfamily, with the most pronounced homologies to gp130 and two other β-type cytokine receptors (19), the receptors for lymphocyte inhibitory factor and granulocyte CSF. Individually, huIL-12Rβ1 and huIL-12Rβ2 expressed in COS-7 cells bind ^{125}I-huIL-12 with a K_d of about 5 nM, corresponding to the low affinity binding seen in PHA-activated lymphoblasts. In addition to this low affinity binding site, cells expressing both huIL-12R subunits exhibit high affinity ^{125}I-huIL-12 binding (K_d = ~50 pM) and IL-12 responsiveness (17, 18).

Bioactive IL-12 is a heterodimer composed of a p40 and a p35 protein subunit. Homodimeric mouse IL-12 p40 subunit (mo(p40)$_2$) is a potent mouse IL-12 antagonist that has been shown to block ^{125}I-huIL-12 binding to huIL-12Rβ1, but not to high affinity IL-12R, on PHA-activated human lymphoblasts (20). Anti-huIL-12Rβ1 mAbs have been shown to specifically inhibit huIL-12-induced proliferation of PHA-activated lymphoblasts, development of LAK activity, and production of IFN-γ from resting PBMC (16), demonstrating that huIL-12Rβ1 is necessary for huIL-12 signaling. In addition, the huIL-12 heterodimer-specific mAb 20C2 has been shown to inhibit huIL-12-induced proliferation of PHA-activated lymphoblasts, but does not inhibit low affinity ^{125}I-huIL-12 binding to huIL-12Rβ1-transfected COS-7 cells (21). The present study demonstrates that IL-12 inhibitors can be grouped into two classes that differ in their ability to block huIL-12 interaction with either huIL-12Rβ1 or huIL-12Rβ2, and that these two classes of IL-12 inhibitors can synergistically inhibit the multiple interactions between IL-12 and its functional receptor complex.

Materials and Methods

Cell lines, proteins, and method of transfection

COS-7 cells were obtained from the American Type Culture Collection (Rockville, MD) and were transfected with pEF-BOS expression constructs (22) encoding human IL-12Rβ1 (15) and/or IL-12Rβ2 (18). In the experiments shown, COS-7 cells (40×10^6) were transfected with 25 μg of IL-12Rβ2-containing and/or 2.5 μg of IL-12Rβ1-containing pEF-BOS DNA by electroporation using a Bio-Rad Electroporator (Bio-Rad Laboratories, Richmond, CA) at 250 μF and 350 V and a 0.4-cm cuvette as previously described (23). The 10:1 ratio of huIL-12Rβ2:huIL-12Rβ1 expression construct DNA was used to help compensate for the greater expression efficiency of huIL-12Rβ1 compared with that of huIL-12Rβ2 (18). Purified recombinant huIL-12 and mo(p40)$_2$ were provided by F. Podlaski and A. Stern (Department of Inflammation/Autoimmune Diseases, Hoffmann-La Roche, Nutley, NJ). Radioiodination of huIL-12 was

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[2] Abbreviations used in this paper: huIL-12, human interleukin-12; mo(p40)$_2$, mouse interleukin-12 p40 subunit homodimer.

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performed using IodoGen (Pierce, Rockford, IL) as previously described (24) and yielded ^{125}I-huIL-12 with a sp. act. of about 4,000 cpm/fmol. The anti-huIL-12Rβ1 mAbs 2B10 and 2-4E6 have been described previously (16). The heterodimer-specific anti-huIL-12 mAb 20C2 has also been previously described (21, 25).

^{125}I-huIL-12 binding assays

Binding assays were conducted using COS-7 cells harvested 48 to 72 h after transfection as described previously (15). Briefly, 1×10^5 COS-7 cells were incubated with various concentrations of ^{125}I-huIL-12 in the absence (total binding) and the presence (nonspecific binding) of 10 μg/ml huIL-12 for 90 min at 22°C. Cell-associated radioactivity was determined by centrifuging the cells through oil and measuring the radioactivity present in the cell pellet. Analysis of the binding data by the method of Scatchard (26) was performed using the nonlinear regression LIGAND equilibrium binding data analysis program (27). The number of receptor sites per cell was calculated assuming that all transfected COS-7 cells were expressing equal numbers of IL-12Rs. Therefore, we are actually reporting an average number of receptor sites per cell. For huIL-12Rβ1, about 90 to 100% of the transfected COS-7 cells appear to express huIL12Rβ1 by flow cytometric analysis with anti-huIL-12Rβ1 mAbs. Anti-huIL-12Rβ2 mAbs are not currently available, and therefore the transfection efficiency for huIL-12Rβ2 in COS-7 cells is not known.

IL-12-induced proliferation and IFN-γ production assays

IL-12-stimulated proliferation was determined using PHA-activated lymphoblasts as described previously (28). All proliferation assays were conducted in triplicate. IFN-γ production by PBMC was measured in the presence of 20 U/ml rIL-2 and 200 U/ml huIL-12 as described previously (29). At these concentrations, neither IL-2 nor IL-12 alone elicited IFN-γ secretion. IFN-γ cultures were conducted in quadruplicate, and IFN-γ was measured using a specific ELISA as previously described (29).

Results

Effects of various IL-12 antagonists on the binding of ^{125}I-huIL-12 to COS-7 cells expressing a single IL-12R subunit

The functional, high affinity IL-12R is composed of at least two protein subunits, huIL-12Rβ1 and huIL-12Rβ2. As a first step in the investigation of the interaction of IL-12 with its receptor complex, the abilities of various inhibitors of IL-12 to interfere with IL-12 binding to transiently transfected COS-7 cells expressing either huIL-12Rβ1 or huIL-12Rβ2 were studied. Both the β1 and β2 receptor subunits, when expressed in COS-7 cells, bound ^{125}I-huIL-12 (Fig. 1) with low affinity (K_d = ~5 nM), in agreement with previously reported results (15, 18). As expected, mAb 2B10, which recognizes huIL-12Rβ1 (16), blocked ^{125}I-huIL-12 binding to COS-7 cells expressing huIL-12Rβ1. Treatment with 2B10 inhibited ^{125}I-huIL-12 binding by 78 ± 5% at all concentrations of ^{125}I-huIL-12 examined. Increasing the concentration of 2B10 did not result in further inhibition of ^{125}I-huIL-12 binding; however, treatment with a combination of two anti-huIL-12Rβ1 mAb, 2B10 and 2-4E6, did result in complete inhibition of ^{125}I-huIL-12 binding to huIL-12Rβ1-transfected COS-7 cells (data not shown). In contrast, 2B10 had no effect on ^{125}I-huIL-12 binding to COS-7 cells expressing huIL-12Rβ2 (Fig. 1). Isotype control Abs had no significant effect on ^{125}I-huIL-12 binding to COS-7 cells expressing huIL-12Rβ1 or huIL-12Rβ2 (data not shown). Similarly, mouse p40 homodimer (mo(p40)$_2$), a known IL-12 antagonist (20, 30, 31), was able to completely inhibit ^{125}I-huIL-12 binding to huIL-12Rβ1-transfected COS-7 cells (Fig. 2) as previously reported (20). Interestingly, mo(p40)$_2$ had no effect on ^{125}I-huIL-12 binding to huIL-12Rβ2-transfected COS-7 cells (Fig. 2). This demonstrates that mo(p40)$_2$ interacts primarily with the β1 chain of the IL-12R complex.

We next examined the ability of 20C2, a heterodimer-specific anti-huIL-12 mAb (21), to block binding of ^{125}I-huIL-12 to the various IL-12R subunits (Fig. 3). In contrast to 2B10 and mo(p40)$_2$, 20C2 increased binding of ^{125}I-huIL-12 to huIL-12Rβ1-transfected COS-7 cells. A similar increase in ^{125}I-huIL-12 binding to huIL-12Rβ1-transfected COS-7 cells was observed when the nonneutralizing mAb 4D6, directed against the huIL-12 p40 subunit (32), was used (data not shown), suggesting that the observed increase in ^{125}I-huIL-12 binding was due to cross-linking

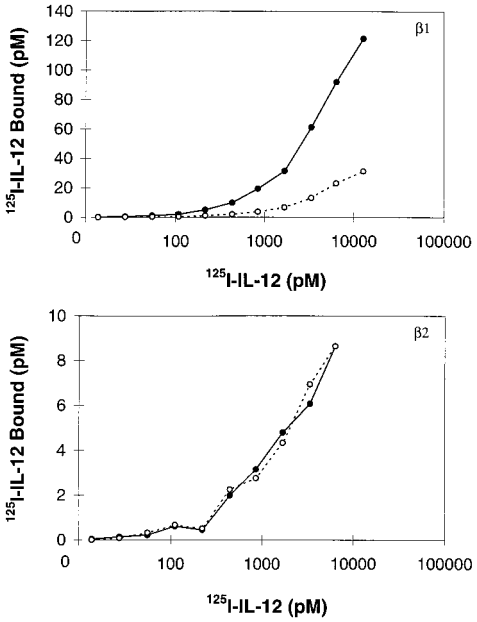

FIGURE 1. Effects of anti-huIL-12Rβ1 mAb 2B10 on ^{125}I-huIL-12 binding to huIL-12R-transfected COS-7 cells. Specific binding of ^{125}I-huIL-12 to huIL-12R-transfected COS-7 cells (huIL-12Rβ1, *upper panel*; huIL-12Rβ2, *lower panel*) was determined in the absence (solid circles) or the presence (open circles) of 25 μg/ml of the anti-huIL-12Rβ1 mAb 2B10 as described in *Materials and Methods*. Similar results were found in three independent experiments, and data from a representative experiment are shown.

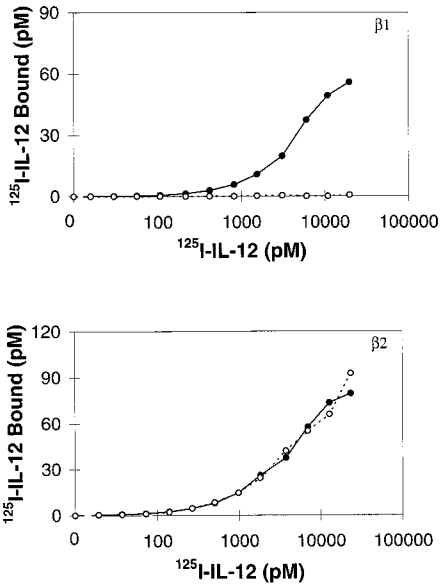

FIGURE 2. Effects of mo(p40)$_2$ on ^{125}I-huIL-12 binding to huIL-12R-transfected COS-7 cells. Specific binding of ^{125}I-huIL-12 to huIL-12R-transfected COS-7 cells (huIL-12Rβ1, *upper panel*; huIL-12Rβ2, *lower panel*) was determined in the absence (solid circles) or the presence (open circles) of 250 ng/ml mo(p40)$_2$ as described in *Materials and Methods*. Similar results were found in three independent experiments, and data from a representative experiment are shown.

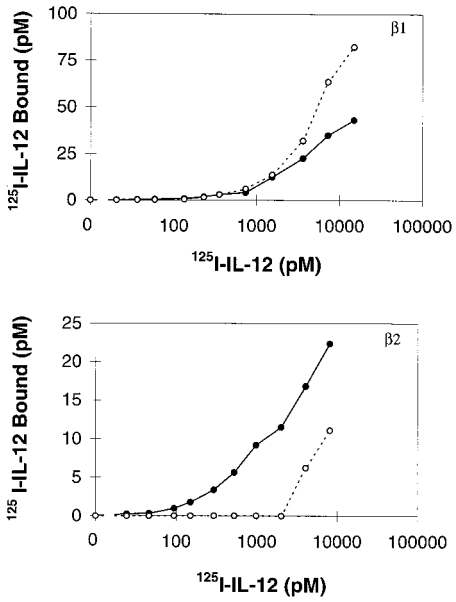

FIGURE 3. Effects of anti-huIL-12 p75 mAb 20C2 on ^{125}I-huIL-12 binding to huIL-12R-transfected COS-7 cells. Specific binding of ^{125}I-huIL-12 to huIL-12R-transfected COS-7 cells (huIL-12Rβ1, *upper panel*; huIL-12Rβ2, *lower panel*) was determined in the absence (solid circles) or the presence (open circles) of 10 μg/ml of the anti-huIL-12 p75 mAb 20C2 as described in *Materials and Methods*. Similar results were found in three independent experiments, and data from a representative experiment are shown.

of ^{125}I-huIL-12 molecules, which then bound to the cell surface. In contrast, 20C2 clearly inhibited the binding of ^{125}I-huIL-12 to huIL-12Rβ2-transfected COS-7 cells, demonstrating that the binding of 20C2 to huIL-12 prevents the interaction of huIL-12 with the β2 receptor subunit.

Effects of various IL-12 antagonists on the binding of ^{125}I-huIL-12 to COS-7 cells expressing both IL-12Rβ1 and IL-12Rβ2 subunits

In agreement with previously reported results (18), coexpression of huIL-12Rβ1 and huIL-12Rβ2 in COS-7 cells creates both high (K_d = 50 pM; 3,000 sites/cell) and low (K_d = 7 nM; 115,000 sites/cell) affinity ^{125}I-huIL-12 binding sites (Fig. 4). Scatchard analysis and nonlinear regression curve fitting of ^{125}I-huIL-12 binding in the presence of mAb 20C2 demonstrated that 20C2 treatment blocked high affinity ^{125}I-huIL-12 binding to the cotransfected cells, leaving the low affinity binding sites (K_d = 8 nM) largely unaffected (Fig. 4). This is in agreement with the previously reported ability of 20C2 to block high affinity, but not low affinity, binding of ^{125}I-huIL-12 to PHA-activated human lymphoblasts (21).[3] Similarly, in three independent experiments, mo(p40)$_2$ treatment blocked high affinity ^{125}I-huIL-12 binding, whereas low affinity ^{125}I-huIL-12 binding (K_d = 1 nM) was blocked 82 ± 8% by mo(p40)$_2$ treatment (Fig. 4). Inhibition of low affinity ^{125}I-huIL-12 binding by mo(p40)$_2$ is presumably due to inhibition of ^{125}I-huIL-12 binding to the large number of huIL-12Rβ1 homodimers present on the cotransfected COS-7 cells (as shown in Fig. 2, *upper panel*), with the residual ^{125}I-huIL-12 binding measured in the presence of mo(p40)$_2$ presumably due to low affinity binding to the relatively few huIL-12Rβ2 homodimers present on the cotransfected COS-7 cell surface. The ability of mo(p40)$_2$ to block high affinity binding to rIL-12R on cotransfected COS-7 cells contrasts with its previously reported (20) inability to inhibit binding of ^{125}I-huIL-12 to naturally occurring, high affinity IL-12R on PHA-activated human lymphoblasts.

In contrast to the complete inhibition of high affinity ^{125}I-huIL-12 binding by 20C2 or mo(p40)$_2$, Scatchard analysis of ^{125}I-huIL-12 binding following treatment of huIL-12Rβ1/β2 cotransfected COS-7 cells with 2B10 demonstrated inhibition of low affinity ^{125}I-huIL-12 binding, with high affinity binding left essentially unchanged (Fig. 4). In a series of three independent experiments, 2B10 treatment (25 μg/ml) blocked 78 ± 6% of low affinity ^{125}I-huIL-12 binding, but had little, if any, effect on high affinity binding to the cotransfected COS-7 cells (24 ± 21% inhibition). Increasing the concentration of 2B10 did not increase its effect on high or low affinity binding (data not shown). Isotype control Abs had no effect on the binding of ^{125}I-huIL-12 to the cotransfected COS-7 cells (data not shown). Similar experiments conducted with PHA-activated human lymphoblasts demonstrated that 2B10 partially inhibited ^{125}I-huIL-12 binding. However, the low levels of binding that remained upon 2B10 treatment made it difficult to evaluate the K_d of the remaining binding sites (data not shown). In contrast, combinations of 20C2 and mo(p40)$_2$ or 20C2 and 2B10 completely blocked binding of ^{125}I-huIL-12 to PHA-activated human lymphoblasts.

[3] R. Chizzonite, T. Truitt, P. Nunes, B. Desai, A. Chua, A. Stern, M. Gately, and U. Gubler. Low and high affinity receptors for IL-12 on human T cells: Characterization by IL-12 and anti-receptor antibody binding. *Submitted for publication.*

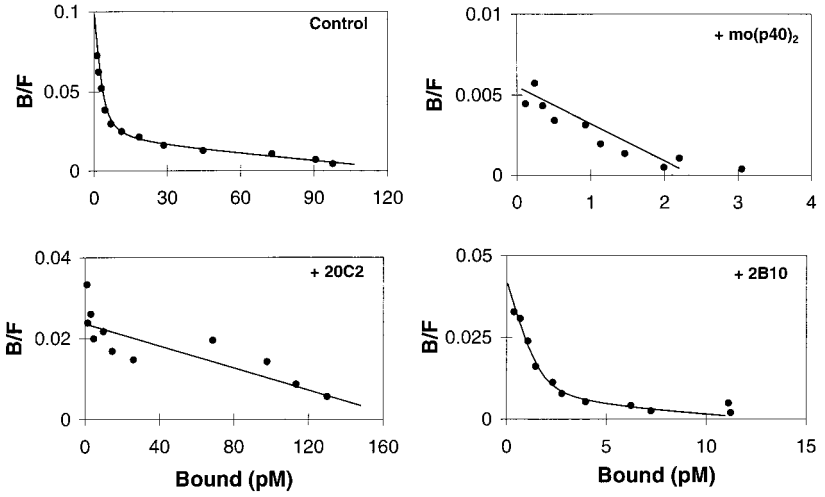

FIGURE 4. Scatchard analysis of ^{125}I-huIL-12 binding to huIL-12Rβ1/β2-cotransfected COS-7 cells. Binding of ^{125}I-huIL-12 to huIL-12Rβ1/β2-cotransfected COS-7 cells was determined as described in *Materials and Methods* in the absence or the presence of 10 μg/ml 20C2, 250 ng/ml mo(p40)$_2$, or 25 μg/ml 2B10 as indicated. Binding data were analyzed using nonlinear regression and were plotted by the method of Scatchard. Binding parameters for the data shown are: control: 50 pM, 3,000 sites/cell; 7 nM, 115,000 sites/cell; 20C2: 8 nM, 160,000 sites/cell; mo(p40)$_2$: 1 nM, 2,300 sites/cell; 2B10: 40 pM, 1,500 sites/cell; 3 nM, 11,000 sites/cell. Similar results were found in three independent experiments, and data from a representative experiment are shown.

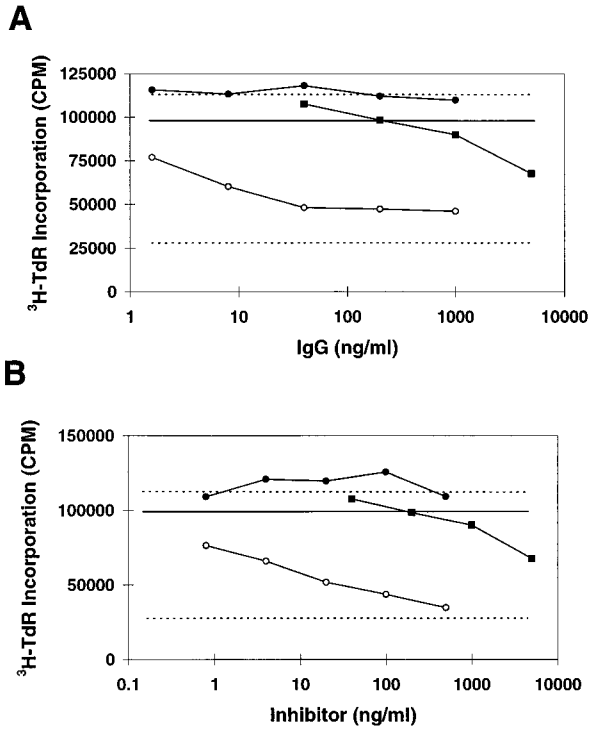

FIGURE 5. Effects of 20C2 and either 2B10 or mo(p40)$_2$ on IL-12-stimulated proliferation of PHA-activated lymphoblasts. PHA-activated lymphoblasts were incubated for 48 h in the presence of 250 pg/ml huIL-12 and the indicated concentration of 2B10 alone (*A*, closed circles), mo(p40)$_2$ alone (*B*, closed circles), 20C2 alone (closed squares), or 200 ng/ml 20C2 added together with either 2B10 (*A*, open circles) or mo(p40)$_2$ (*B*, open circles). Lymphoblast proliferation was assessed by measurement of [^{3}H]TdR incorporation during a final 6-h period as described in *Materials and Methods*. The lower and upper dotted lines represent the amount of [^{3}H]TdR incorporated in cultures lacking Abs in the absence and the presence of 250 pg/ml IL-12, respectively, and the solid line represents the amount of [^{3}H]TdR incorporated in the presence of 250 pg/ml IL-12 and 200 ng/ml 20C2. Similar results were found in three independent experiments, and data from a representative experiment are shown.

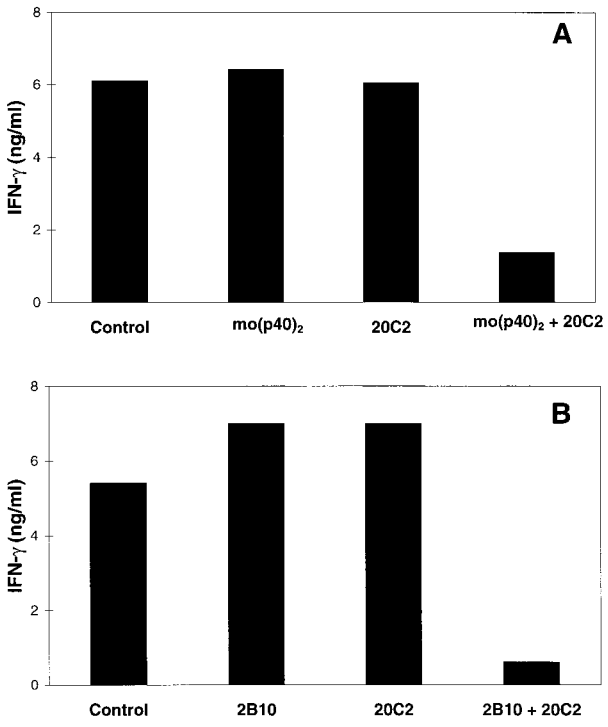

FIGURE 6. Effects of 20C2 and either mo(p40)$_2$ or 2B10 on IL-12-stimulated IFN-γ production by PBMC. PBMC were isolated and stimulated with 20 U/ml rIL-2 and 1 ng/ml huIL-12 in the absence or the presence of 20C2 and/or either mo(p40)$_2$ or 2B10 as indicated. *A*, 20 ng/ml 20C2 and/or 500 ng/ml mo(p40)$_2$; *B*, 50 ng/ml 20C2 and/or 25 μg/ml 2B10. IFN-γ production was determined as described in *Materials and Methods*. IFN-γ was not detected in the absence of IL-12 stimulation. Similar results were found in at least two independent experiments, and data from a representative experiment are shown.

Inhibition of IL-12-induced proliferation and IFN-γ production

The abilities of the various IL-12 inhibitors, alone and in combination, to block IL-12 bioactivity were examined next. IL-12-induced proliferation of PHA-activated lymphoblasts is a commonly used measure of IL-12 bioactivity (28). Consistent with its failure to inhibit high affinity IL-12R binding, 2B10 alone was a poor inhibitor of IL-12-induced proliferation (Fig. 5*A*), in agreement with previous results (16). Although 20C2 alone was able to inhibit IL-12-induced proliferation, the combination of 20C2 with 2B10 acted synergistically to potently inhibit IL-12 bioactivity (Fig. 5*A*). Similarly, the combination of mo(p40)$_2$ and 20C2 synergistically inhibited IL-12-induced lymphoblast proliferation (Fig. 5*B*). Control mouse and rat Abs showed no significant effect on IL-12-induced lymphoblast proliferation (16).

IL-12 plays an important role in stimulating the production of IFN-γ (6). Therefore, we investigated the effects of the various classes of inhibitors on IL-12-induced IFN-γ production from human PBMC. In the absence of IL-12 stimulation, IFN-γ was not detected. Whereas 500 ng/ml mo(p40)$_2$ and 20 ng/ml 20C2 alone each had no effect on IL-12-stimulated IFN-γ production, the combination of these two inhibitors synergistically decreased IFN-γ production by 80% (Fig. 6*A*). Similar synergistic inhibitory properties of 2B10 and 20C2 were observed (Fig. 6*B*), demonstrating that the two classes of IL-12 antagonists can synergistically inhibit both IL-12-induced proliferation and IFN-γ production. Control mouse and rat Abs showed no significant effect on IL-12-induced IFN-γ production (16).

Discussion

Functional, high affinity receptors for IL-12 have been described on PHA-activated human lymphoblasts and the human T cell line Kit225/K6 (13–16). We have recently described the identification of two cDNAs encoding IL-12R subunits, designated huIL-12Rβ1 and huIL-12Rβ2, that in combination can confer high affinity IL-12 binding and IL-12 responsiveness (17, 18). In this study we have investigated the effects of various inhibitors of IL-12 on its interaction with these IL-12R proteins. Three IL-12 inhibitors were examined in this work.

The mAb 20C2 has been shown to be largely specific for the human IL-12 p75 heterodimer (25, 32), but has also been reported to react weakly with the IL-12 p40 subunit (25). We have found that the 20C2 mAb can interact weakly with the human p40 homodimer, but did not observe binding to purified human p40 monomer. Moreover, the p35 subunit also appears to contribute to the epitope recognized by 20C2 (M. Gately and P. Ling, unpublished observations). Previous studies have shown that 20C2 inhibits IL-12-induced proliferation of PHA-activated human lymphoblasts without inhibiting low affinity binding of ^{125}I-huIL-12 to huIL-12Rβ1-transfected COS-7 cells (21).

In addition to 20C2, we used mAb 2B10 to examine IL-12/IL-12R interactions. We have previously reported that 2B10 recognizes huIL-12Rβ1, and although by itself it is not inhibitory, the

combination of anti-huIL-12Rβ1 mAbs 2-4E6 and 2B10 inhibits IL-12-induced proliferation of PHA-activated lymphoblasts (16). Extension of previous studies of mouse p40 homodimer, which demonstrated potent inhibition of the biologic activities of mouse IL-12-induced, but not huIL-12-induced, proliferation of PHA-activated human lymphoblasts (20), was also conducted.

Using transfected COS-7 cells expressing either huIL-12Rβ1 or huIL-12Rβ2, two classes of IL-12 inhibitors were identified based on their ability to interfere with the binding of ^{125}I-huIL-12 to these receptor subunits. mAb 2B10 and mo(p40)$_2$ blocked binding to huIL-12Rβ1, but not to huIL-12Rβ2. This extends the previous observation that mo(p40)$_2$ interacts primarily with the IL-12Rβ1 subunit of either the human (20) or mouse (33) IL-12R by demonstrating that mo(p40)$_2$ does not interact strongly with the huIL-12Rβ2 subunit. Whereas both mo(p40)$_2$ and 2B10 interfere with the interactions between huIL-12 and huIL-12Rβ1, mo(p40)$_2$ appears to be a more effective inhibitor, since 2B10 can only inhibit about 80% of the low affinity binding of ^{125}I-huIL-12 to huIL-12Rβ1-transfected COS-7 cells, whereas mo(p40)$_2$ completely blocks low affinity binding to huIL-12Rβ1.

In contrast to mo(p40)$_2$ and 2B10, anti-huIL-12 heterodimer-specific mAb 20C2 recognizes an epitope on huIL-12 that interacts with huIL-12Rβ2 but not huIL-12Rβ1, as demonstrated by the selective inhibition of ^{125}I-huIL-12 binding to huIL-12Rβ2-transfected COS-7 cells. At lower huIL-12 concentrations ($<$2 nM), 20C2 completely blocks this low affinity binding.

Studies using huIL-12Rβ1/β2-cotransfected COS-7 cells support the hypothesis that direct interaction of IL-12 with both receptor subunits is required for high affinity IL-12 binding. Inhibition of ^{125}I-huIL-12 interaction with either the β1 or the β2 subunit by mo(p40)$_2$ or 20C2, respectively, eliminated the observed high affinity ^{125}I-huIL-12 binding. Low affinity binding, however, presumably representing interaction of ^{125}I-huIL-12 with the uninhibited receptor subunit, remained. Simultaneous treatment with both types of inhibitors, e.g., 20C2 and mo(p40)$_2$, completely inhibited ^{125}I-huIL-12 binding (data not shown). In agreement with the lower efficacy of 2B10 to inhibit the huIL-12/huIL-12Rβ1 interactions discussed above, treatment with 2B10 was unable to interfere with high affinity binding to the cotransfected cells, although low affinity binding was decreased by 2B10 treatment. The lower efficacy of 2B10 to block huIL-12/huIL-12Rβ1 interactions can also explain why a combination of anti-huIL-12Rβ1 mAb 2B10 and 2-4E6 is required to inhibit huIL-12 bioactivity (16). Consistent with the important roles of IL-12 binding to both the β1 and β2 receptor subunits, the two classes of IL-12 inhibitors can act synergistically to inhibit IL-12 bioactivity. Combinations of the huIL-12Rβ1 inhibitor mo(p40)$_2$ or 2B10 and the huIL-12Rβ2 inhibitor 20C2 synergistically inhibit IL-12-stimulated PHA-activated human lymphoblast proliferation and IL-12-induced IFN-γ production from human PBMC.

It is interesting to note that whereas mo(p40)$_2$ can inhibit high affinity binding of ^{125}I-huIL-12 to huIL-12Rβ1/β2-cotransfected COS-7 cells, mo(p40)$_2$ is not a potent inhibitor of high affinity binding of ^{125}I-huIL-12 to PHA-activated human lymphoblasts or of PHA-activated human lymphoblast proliferation (20). In addition, the high affinity binding of ^{125}I-huIL-12 to cotransfected COS-7 cells has a K_d of about 50 pM (18), whereas PHA-activated human lymphoblasts and the human Kit225/K6 cell line exhibit high affinity ^{125}I-huIL-12 binding with a K_d of about 5 to 20 pM (15) (R. Chizzonite, unpublished observations). This suggests that cotransfected COS-7 cells do not exactly mimic the native high affinity IL-12R found on activated T or NK cells. In agreement with the idea that differences exist between cells expressing the native IL-12R and cells expressing cotransfected β1 and β2 receptor subunits, cotransfected Ba/F3 cells expressing huIL-12Rβ1 and huIL-12Rβ2 are IL-12 responsive (17, 18), and mo(p40)$_2$ is a potent inhibitor of huIL-12-induced proliferation in these cotransfected Ba/F3 cells (D. H. Presky and M. K. Gately, unpublished observations). It is still unknown whether these differences are due to an additional, as yet unidentified, component of the high affinity IL-12R complex on PHA-activated lymphoblasts or to differences in protein processing in the various cell types.

In conclusion, we have identified two classes of IL-12 antagonists that differentially inhibit the interaction of IL-12 with the IL-12Rβ1 and IL-12Rβ2 subunits of the high affinity IL-12R complex. These two classes of IL-12 inhibitors can function to synergistically block IL-12-stimulated proliferation and IFN-γ production. Overall, the results suggest that binding of IL-12 to the high affinity IL-12R complex involves multiple interaction sites, including direct interactions with both the β1 and β2 receptor subunits. IL-12 appears to interact with huIL-12Rβ1 primarily via domains on the IL-12 p40 subunit and with huIL-12Rβ2 via a heterodimer-specific region of IL-12 to which the IL-12 p40 and p35 subunits may both contribute. We are currently exploring whether simultaneous inhibition of the multiple sites of interaction between IL-12 and its receptor complex can be exploited to design potent IL-12 antagonists.

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