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IL-12 is a heterodimeric cytokine produced by APC that promotes the development of CD4$^+$ Th1 cells and their IFN-$\gamma$ production after TCR/CD3 triggering. We here investigated the capacity of IL-12 to modify the expression on T cells of CD40 ligand (CD40L or CD154), a molecule transiently expressed on activated T cells and known to be of utmost importance for cognate interaction with B cells and for activation of dendritic cells and macrophages. Our data demonstrate that IL-12 up-regulates CD40L expression on anti-CD3-activated human peripheral blood T cells. For optimal induction of CD40L, IL-12 synergizes with IL-2 as well as with other costimulatory interactions, such as B7/CD28. The effect of IL-12 was observed at both the protein and the mRNA level. T cells costimulated by IL-12 provided more efficient help for IL-4-dependent B cell proliferation and for IgG production than when activated in the absence of IL-12. This helper activity was blocked by an mAb against CD40L, indicating that the effect of IL-12 on B cells is mediated indirectly through CD40L. The data thus suggest that the effects of IL-12 on cellular and humoral immune responses are partly mediated through CD40L induction. The Journal of Immunology, 1998, 160: 1166–1172.

Materials and Methods

Isolation of peripheral blood T cells

All subjects donating blood for this study were healthy volunteers of both sexes, aged 20 to 50 yr. PBMC were isolated from heparinized venous blood on Ficoll-Hypaque density gradients. After washing, the cells were resuspended at a concentration of 5 x 10$^5$ cells/ml in complete culture medium consisting of RPMI 1640 (Boehringer Ingelheim, Heidelberg, Germany) supplemented with 2 mM l-glutamine, penicillin (100 U/ml), streptomycin (100 $\mu$g/ml), and 10% iron-supplemented bovine calf serum (HyClone, Logan, UT). Monocytes were removed by cold agglutination by rotating tubes for 30 min at 4°C. The agglutinated monocytes were sedimented out, and the lymphocytes were further purified using Lymphoquick-T (One Lambda, Inc., Los Angeles, CA) supplemented with complement-fixing anti-NK (anti-Leu 11b (anti-CD16), anti-Leu 7 (anti-FcRIII, anti-CD16), anti-Leu 12 (anti-FcRII) and Fc$^+$RI. The resulting T cell preparations contained >99% CD3$^+$ and <1% CD16$^+$ cells; CD14$^+$ monocytes and HLA-DR$^+$ cells were not detected. These cells could not be activated by PHA or rIL-2 alone (10 U/ml).

Cell lines

The P815 cell line is an NK-resistant DBA/2 derived murine mastocytoma cell line that expresses mouse Fc$\gamma$RII and Fc$\gamma$RIII. The P815/CD80 cell line, transfected with human CD80 cDNA, was a gift from L. E. Lanier (DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA) (33). The 3T6 mouse fibroblast cell line, transfected with CD40L, was a gift from K. Thielemans (Free University of Brussels, Brussels, Belgium). The P815 cells and the 3T6 cells were cultured in complete culture medium supplemented with gentamicin (50 $\mu$g/ml), sodium pyruvate (1 mM/ml), nonessential amino acids (1/100), 50 $\mu$M 2-ME, and 10% FCS (Gibco, Paisley, Scotland). Geniticin (1 mg/ml) was added every 1 to 2 wk

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3 Abbreviations used in this paper: CD40L, CD40 ligand; DC, dendritic cells.
for selecting transfected cells. The cells were given fresh medium every 2 or 3 days.

**Isolation of tonsillar B and T lymphocytes**

Fresh tonsils were obtained from children undergoing tonsillectomies. The tissue was dispersed by cutting with scalpel blades. Fragments were allowed to pass through a filter, and cell suspensions were collected. B cells were prepared by two cycles of rosetting with AET-treated SRBC and removal of E-rosetting cells on Ficol-Hypaque density gradients. T cells were prepared by two cycles of Lymphoprik-T (One Lambda Inc., Canoga Park, CA). The resultant B cell preparations contained 95% CD20^+ cells, and the tonsillar T cell preparations contained 97% CD3^+ cells as determined by FACS analysis.

**Cytokines and mAbs**

Recombinant IL-12 was a gift from Genetics Institute (Cambridge, MA). Recombinant IL-4 was obtained from Innogenetics (Gent, Belgium). Anti-CD3 mAb UCHT-1 (IgG1) was a gift from Dr. P. Beverley, Imperial Research Cancer Fund (London, U.K.). The clones producing anti-CD3 mAb OKT3 and anti-CD11b OKM1 were obtained from American Type Culture Collection (Rockville, MD). Anti-CD28 mAb 9.3 was a gift from C. June (Naval Medical Research Institute, Bethesda, MD). Anti-CD2 mAb 9.6 (IgG2a) was purchased from Oncogen (Seattle, WA), and mAb 9–1 was purchased from XOMA Corp. (Berkeley, CA). mAb 9.6 blocks E rosette formation and is directed against the CD58-binding epitope. The mAb 9–1 detects the CD2 neoepitope on activated T cells. Humanized anti-Tac mAb (directed at the p55 chain of the human lymphocyte IL-2R [CD25]) and humanized MiiK81 (a mAb to the p75 chain of the IL-2R [CD22]) were gifts from Dr. J. Hakimi (Hoffmann-La Roche, Nutley, NJ). A neutralizing anti-IL-2 mAb (B-05) was purchased from Innogenetics (Besançon, France). Anti-IL-10R mAb (clone 37607.11) was purchased from R&D Systems (Minneapolis, MN). A neutralizing anti-IFN-γ mAb (9D910) was a gift from Dr. M. de Ley (Department of Biochemistry, Catholic University of Leuven, Leuven, Belgium). Anti-CD40L mAb M90 (IgG1) (34) was a gift from W. Fanslow (Immunex Corp., Seattle, WA). Anti-CD40L-FTC (clone 24-31) was obtained from Ancell Corp. (Bayport, MN).

**Cell culture conditions and FACS analysis for CD40L expression**

For stimulation with immobilized anti-CD3, wells were first coated with OKT3 (final concentration, 1 µg/ml) and then washed three times with PBS. Purified peripheral blood T cells (5 × 10^5) were cultured either on OKT3-coated plates or with anti-CD3 mAb UCHT-1 (1 µg/ml) bound to FcsYr on mitomycin C-treated P815 cells (1 × 10^6). All cultures were set up in a 1-ml volume in the presence or the absence of rIL-12 (1 ng unless otherwise indicated) in 24-well culture plates. CD80 (transfected into P815 cells; 1 × 10^5/ml), soluble anti-CD28 mAb 9.3 (0.5 µg/ml), or a pair of anti-CD2 mAbs (9-1 (2 µg/ml) and 9.6 (2 µg/ml) were included in the above cultures to provide a second signal. The culture plates were incubated in a 37°C, humidified atmosphere of 5% CO₂. After 16, 40, and 64 h of incubation, the cell pellets were stained with anti-CD40L-FITC mAb or isotype-matched negative control Ab for 30 min at 4°C. Following two washes with PBS, the cells were fixed in 1% paraformaldehyde in saline and analyzed on a FACSort flow cytometer (Becton Dickinson, Mountain View, CA).

**RNA isolation and reverse transcription-PCR**

T cell cultures were harvested 4 or 16 h after the initial stimulation as described above. Approximately 5 × 10^6 stimulated T cells were pelleted and directly used for RNA isolation. Total RNA was isolated by the gua- nidium isothiocyanate method (35) and quantified spectrophotometrically. cDNA was prepared from 2.5 µg of total RNA using the SuperScript reverse transcriptase preamplification system (Life Technologies, Grand Island, NY) with oligo(dT) primer. The cDNA samples were then subjected to PCR analysis using the following primers: 5'-AGATGTTGTTTTACTGCTGGC-3' and 5'-ACTACATACAACTAACTTCC-3' for detection of CD40L, and 5'-GGCTACCATAGGATGATAGTGCGCC-3' and 5'-GGATGCTCTTCTTCTGCGGC-3' for detection of β-actin as a cDNA quality control. Samples were amplified by Goldstar Taq polymerase (Eurogentec, Seraing, Belgium) by 24 cycles of denaturation at 94°C for 30 s, annealing at 60°C (CD40L) or at 56°C (β-actin) for 30 s, and extension at 72°C for 1 min. PCR products were analyzed by electrophoresis on 1% agarose gels and visualized by ethidium bromide staining.

**Table 1.** rIL-12 up-regulates CD40L expression on anti-CD3-activated T cells

<table>
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<th>Culture Addition^a</th>
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<tr>
<td>Resting T</td>
<td>Anti-CD3</td>
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<td></td>
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<td>&lt;10</td>
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^a Purified T cells (5 × 10^5/ml) were stimulated with immobilized anti-CD3 mAb OKT3 in the presence or absence of rIL-12 (1 ng/ml), either parental or CD80 transfected, in the presence or the absence of rIL-12 (1 ng/ml) in 96-well flat-bottom microculture plates. Recombinant IL-4 (100 ng/ml) was added to all cell cultures. Anti-IL-2 (2 µg/ml) and anti-IL-2R mAbs (1 µg/ml for each) were also added to block the effects of endogenous IL-2 on CD40L expression and B cell proliferation. The final volume of each cell culture was 200 µl. After 5 days, cells were pulsed with 1 µCi of [³H]thymidine. Eight hours later, cells were harvested, and thymidine incorporation was measured using a beta counter.

^b After 40 h, cells were incubated with either anti-CD40L-FTC or isotype matched mouse IgG and analyzed on a FACSort with the Lysis II software (Becton Dickinson). The results are expressed as mean of mean fluorescence intensity (MFI) ± SEM from six independent experiments. *p < 0.05.

**Analysis of Th cell activity for B cell proliferation and IgG production**

Tonsillar B cells (1 × 10^7/well) and tonsillar T cells (irradiated, 3000 rad, 10^7/well) were cultured with soluble anti-CD3 mAb UCHT-1 (1 µg/ml) and mitomycin C-treated P815 mouse mastocytoma cells (1 × 10^6/ml), either parental or CD80 transfected, in the presence or the absence of rIL-12 (1 ng/ml) in 5-ml snap-cap Falcon tubes (Becton Dickinson, Lincoln Park, NJ) at 37°C in 5% CO₂. The final volume of each cell culture was 1 ml. After 7 days, cells were spun down, and cell-free supernatant was harvested. The concentrations of human IgG were estimated by ELISA as previously described (9).

**Statistical analysis**

Statistical analysis was performed with the Wilcoxon test for paired samples, p < 0.05 was considered significant.

**Results**

IL-12 up-regulates CD40L expression on anti-CD3-activated T cells

Different concentrations of rIL-12 were added to cultures of purified T cells stimulated with immobilized anti-CD3 mAb OKT3, and cells were then stained for CD40L expression. CD40L was not detected on resting T cells or T cells incubated with rIL-12 alone (data not shown). The expression of CD40L was weakly induced by anti-CD3 stimulation and significantly enhanced by rIL-12 (Table 1). Figure 1 shows that increasing doses of rIL-12 progressively enhanced the expression of CD40L on anti-CD3-activated T cells. The optimal concentration of rIL-12 was 1 ng/ml. The highest CD40L expression on anti-CD3-activated T cells in the presence of rIL-12 occurred after a 40-h cell culture, with a decline of CD40L expression thereafter (Fig. 2).

To study whether IL-12 interacts with other helper signals, purified T cells were cultured with immobilized anti-CD3 mAb OKT3 as the primary signal and with soluble anti-CD28 mAb or a pair of anti-CD2 mAbs (9-1 and 9.6) as helper signals. As shown in Figure 2, addition of anti-CD28 mAb or anti-CD2 mAbs enhanced CD40L expression, but rIL-12 still further augmented and prolonged the expression of CD40L, again with maximal CD40L expression after 40 h of culture.

IL-12 enhances CD40L expression on human T cells when costimulated with CD80

In previous studies we showed that the binding of CD28 on T cells to its natural ligand CD80 on APC in the presence of anti-CD3
mAb enhances and prolongs CD40L expression (9). We therefore tested whether CD80 and IL-12 would synergize in inducing CD40L. To this end, we used mouse mastocytoma P815 cells transfected with CD80 and coated with anti-CD3 (via FcR binding) to stimulate T cells. As shown in Figure 3 and Table II, up-regulation of CD40L induction by CD80 was confirmed. IL-12 did not influence CD40L expression when T cells were stimulated with anti-CD3 on P815 cells. When, however, T cells were cultured with CD80-transfected P815 cells and anti-CD3 mAb, rIL-12 strongly enhanced CD40L expression, thus pointing to a synergy between CD80 and IL-12 for induction of CD40L.

**CD40L expression is regulated through IL-2-dependent and IL-2-independent mechanisms**

Since IL-12 induces IFN-γ and IL-10 production by T cells (24, 36), we asked whether CD40L induction by IL-12 might be mediated indirectly through these other cytokines. To this end, a blocking mAb against the IL-10R or a neutralizing anti-IFN-γ mAb was added to the cultures. Neither anti-IL-10R nor anti-IFN-γ mAb affected the induction of CD40L expression by IL-12 (data not shown). Since endogenous IL-2 plays an important role in the induction of CD40L on T cells (37), we also evaluated whether IL-12 requires the presence of IL-2 to enhance CD40L. T cells were stimulated with soluble anti-CD3 mAb cross-linked by either parental P815 cell FcR or CD80-transfected P815 cells. To parallel cultures, a neutralizing anti-IL-2 mAb and two different anti-IL-2R (anti-Tac and Mikb1) mAbs were added to block all IL-2 activity (38). As shown in Figure 3, anti-IL-2 mAb and anti-IL-2R mAbs strongly, but not completely, reduced CD40L expression on T cells cultured with either anti-CD3 mAb plus CD80-transfected cells. Importantly, rIL-12 still enhanced CD40L expression when IL-2 activity was blocked, indicating that CD40L induction by rIL-12 is IL-2 independent. Together, the data indicate that both IL-2-dependent and IL-2-independent signals (CD80 and IL-12) are involved in CD40L regulation.

**Analysis of CD40L mRNA expression in purified human T cells costimulated by IL-12**

Next, we studied the effect of IL-12 on the expression of CD40L mRNA in activated T cells. T cells were incubated for 4 or 16 h...
with mitomycin C-treated wild-type P815 cells or CD80-transfected P815 cells in the presence or the absence of rIL-12. Soluble anti-CD3 mAb was added as a primary signal. Total RNA was isolated, and reverse transcription-PCR reactions using specific primers for CD40L and β-actin were performed. As shown in Figure 4, no CD40L mRNA was detected in T cells cultured with P815 cells alone (lane 5) or in P815 cells and anti-CD3 mAb in the presence (lane 7) or the absence (lanes 2 and 6) of rIL-12 in either 4- or 16-h cell cultures. However, CD80-transfected P815 cells induced early expression (at 4 h) of CD40L mRNA (lane 3) in anti-CD3 activated T cells. Interestingly, the addition of rIL-12 did not enhance CD40L mRNA at 4 h of stimulation (lane 4), but clearly enhanced CD40L mRNA expression at 16 h of stimulation (lane 9). Thus, IL-12 seems to have a late effect on CD40L mRNA.

T cells costimulated by IL-12 provide more efficient help for B cell proliferation and IgG production via an effect on CD40L-CD40 interaction

We studied the functional relevance of IL-12-induced CD40L expression by analyzing the effect of IL-12 on T cell-dependent B cell proliferation and IgG production. This Th cell activity has been shown to be CD40L dependent (7, 8). Tonsilar B cells were cultured with rIL-4 and irradiated autologous T cells. B cell proliferation was measured by [3H]thymidine incorporation. As shown in Figure 5, no significant B cell proliferation was found in the cultures where B cells were cocultured with T cells stimulated with anti-CD3 mAb and P815 cells. However, B cell proliferation was induced in the presence of CD80-costimulated T cells, and this was further enhanced by rIL-12. When anti-CD40L mAb was added, B cell proliferations were completely blocked. A control mAb did not show any effect on B cell proliferation. In the experiments shown in Figure 5, IL-2 activity was blocked with mAb to IL-2 and to IL-2R α- and β-chains, because IL-2 also strongly influences CD40L expression. However, similar data were obtained when IL-2 activity was not blocked (data not shown). To exclude a direct effect of IL-12 on B cells, B cells were cultured with 3T6 mouse fibroblasts transfected with CD40L and exogenous rIL-4 in the presence or the absence of rIL-12. Recombinant IL-12 did not have any effect on B cell proliferation in this system (data not shown). Thus, the effect of IL-12 on B cell proliferation

Table II. rIL-12 enhances CD40L expression on T cells costimulated by CD80

<table>
<thead>
<tr>
<th>Culture Additiona</th>
<th>Mean Fluorescence Intensityb</th>
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<tbody>
<tr>
<td>Resting T</td>
<td>&lt;10</td>
</tr>
<tr>
<td>P815 + anti-CD3</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>P815 + anti-CD3 + rIL-12</td>
<td>35 ± 7</td>
</tr>
<tr>
<td>P815/CD80 + anti-CD3</td>
<td>187 ± 35</td>
</tr>
<tr>
<td>P815/CD80 + anti-CD3 + rIL-12</td>
<td>370 ± 61</td>
</tr>
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</table>

a T cells (5 × 10^6/ml) were cultured with mitomycin C-treated P815 cells or CD80-transfected P815 cells in the presence or absence of rIL-12 (1 ng/ml) in flat-bottom 24-well culture plates. Anti-CD3 mAb UCHT-1 was added at a concentration of 1 μg/ml.
b After 40 h, cells were incubated with either anti-CD40L-FITC or isotype-matched mouse IgG and analyzed on a FACSort. The results are expressed as mean of mean fluorescence intensity (MFI) ± SEM from six independent experiments.

p < 0.05.

FIGURE 3. Recombinant IL-12 up-regulates CD40L expression through an IL-2-independent mechanism. T cells (5 × 10^6/ml) were stimulated with anti-CD3 mAb UCHT-1 (1 μg/ml) presented on mitomycin C-treated P815 cells or CD80-transfected P815 cells (1 × 10^6/ml) in the presence or the absence of rIL-12 (1 ng/ml). A neutralizing anti-IL-2 mAb (2 μg/ml) in combination with anti-IL-2R mAbs (anti-Tac and Mikβ1; 1 μg/ml for each) were added from the beginning to some cultures as indicated. After 40 h, cells were stained with either anti-CD40L-FITC or isotype-matched mouse IgG and analyzed on a FACScan. Cell number is on the y-axis, and fluorescence intensity in arbitrary units on a log scale is on the x-axis. The number above each histogram represents the mean fluorescence intensity (MFI) of the anti-CD40L-stained cells. The MFI of the isotype control in each histogram is <25. Histograms are from a single experiment and are representative of three experiments with similar results.

FIGURE 4. Recombinant IL-12 enhances CD40L mRNA expression in T cells stimulated by anti-CD3 and CD80. Purified human T cells (1 × 10^6/ml) were cultured for 4 (lanes 2–4) or 16 h (lanes 5–9) before mRNA extraction. Cultures were performed with mitomycin C-treated P815 cells and anti-CD3 mAb in the absence (lanes 2 and 6) or the presence (lane 7) of rIL-12 (1 ng/ml) or with CD80-transfected P815 cells and anti-CD3 mAb in the presence (lanes 3 and 8) or presence (lanes 4 and 9) of rIL-12 (1 ng/ml). Lane 5 represents the T cells (1 × 10^6/ml) that were cultured with mitomycin C-treated P815 cell alone (without anti-CD3 or rIL-12) for 16 h. Total RNA was isolated by the guanidium isothiocyanate method and converted to cDNA. The cDNA was used as template for PCR using CD40L primers and as a control β-actin primers. The CD40L and β-actin PCR products were verified after gel electrophoresis and ethidium bromide staining. The top panel represents the CD40L PCR products, and the bottom panel represents the β-actin PCR products.
most likely proceeds via modulation of the interaction of CD40L on anti-CD3 activated T cells and of CD40 on B cells.

To study effects of IL-12 on Ig production by B cells, we cultured tonsillar mononuclear cells with soluble anti-CD3 mAb and mitomycin C-treated P815 cells, either parental or CD80 transfected, in the presence or the absence of rIL-12. As shown in Figure 6, large amounts of IgG were produced when T cells were stimulated with anti-CD3 mAb presented on P815/CD80 cells. Interestingly, the addition of rIL-12 further enhanced IgG production. Blocking experiments with anti-CD40L mAb resulted in partial inhibition of IgG production by B cells. With relevance to the effects of IL-12, anti-CD40L inhibited IgG production in the presence of rIL-12 to a similar extent as in the absence of IL-12.

Discussion

In the present study we have shown that IL-12 up-regulates the expression of CD40L on anti-CD3-stimulated T cells at both the protein and the mRNA level. This effect is most clear when T cells are costimulated with CD80. Functional studies confirmed that costimulation of T cells with IL-12 results in more efficient help for B cell growth and IgG production, and that this helper activity is dependent on the interaction of CD40L on T cells and of CD40 on B cells.

Requirements for optimal CD40L induction on T cells are incompletely understood. CD40L is not expressed on resting T cells. CD3-TCR triggering or ionomycin alone is sufficient to induce some CD40L expression (6, 39, 40). CD40L can be up-regulated by PMA (39, 40), and endogenous IL-2 production was found to be important for the expression of CD40L (37). Moreover, we have previously reported that ligation of B7 with CD28 strongly up-regulates and prolongs CD40L expression on anti-CD3-activated T cells (9). Roy et al. reported that Ag-induced expression of CD40L on TCR transgenic T cells was inhibited by Abs to class II MHC, CD4, and LFA-1, but not by CTLA-4 Ig, anti-B7-1, or anti-B7-2 (41). Ding et al. reported that induction of CD40L is only partially inhibited by CTLA-4 Ig, and that CD40L could be induced on CD4+ T cells from CD28-deficient mice (42). Thus, B7-independent costimulatory mechanisms are also involved in enhancing the expression of CD40L.

IL-12, as shown in this study, is such a helper signal that up-regulates CD40L expression. Although IL-12 greatly induces IFN-γ and IL-10 production (24, 36), it is unlikely that induction of CD40L by IL-12 is an indirect effect of IFN-γ or IL-10, since a neutralizing anti-IFN-γ mAb or anti-IL-10 mAb did not block the effect of IL-12 on CD40L expression. Moreover, exogenous rIL-10 inhibited CD40L expression (our unpublished observations), and IFN-γ has been shown by others to inhibit the expression of CD40L in mice (40). The inhibition of IFN-γ on CD40L expression was not confirmed in the human system (our unpublished observations). Although endogenous IL-2 plays an important role in the induction of CD40L (37), it is not likely that induction of CD40L by IL-12 is mediated through IL-2. First, our data (43) as well as other reports (44) showed that IL-12 does not affect IL-2 production. Second, IL-12 still enhanced CD40L expression in the presence of a neutralizing anti-IL-2 mAb and anti-IL-2R mAbs that have previously been shown to block all IL-2 activity (38). On the other hand, it is clear from these experiments that IL-12 synergizes with CD80 and IL-2 in inducing CD40L. Therefore, optimal induction of CD40L occurs when IL-12, CD80, and IL-2 are all present to signal the T cells.

The signal transduction mechanism by which IL-12 induces CD40L requires further investigation. It is also not clear yet whether IL-12 has an effect on the transcription of the CD40L gene or on CD40L mRNA stability in activated T cells. IL-12 is the only
cytokine identified to date that can induce phosphorylation of STAT4 (45, 46). In STAT4-deficient mice, all IL-12 functions tested were disrupted, including the induction of IFN-γ, mitogenesis, enhancement of NK cytolytic function, and Th1 differentiation (47, 48). These results demonstrate that STAT4 is essential for mediating responses to IL-12. It will therefore be of major interest to study binding of phosphorylated STAT4 to the promoter region of the CD40L gene.

Another interesting point in our experimental results is the synergy between CD80 and IL-12 in inducing CD40L. Synergistic effects between both helper signals have also been found for IFN-γ and IL-10 production (43, 49). IL-12R chains are expressed by activated T and NK cells. Two IL-12R chains, termed β1 and β2, have been identified to date. The IL-12R β1 subunit is expressed in both Th1 and Th2 cells, while the IL-12R β2 subunit is selectively lacking in Th2 cells (50, 51). Recent reports showed that anti-CD28 mAb and IL-2 augment the expression of IL-12R β1 and induce high affinity IL-12 binding (which correlates with β2-chain expression) to anti-CD3-activated T cells (52). Lack of high affinity IL-12 binding can explain why IL-12 had no effect on CD40L expression when T cells were stimulated with P815 plus anti-CD3 mAb alone. When costimulated with CD80, the strong effects of IL-12 on T cell CD40L expression most likely result from induction of high affinity IL-12R expression.

To confirm that the effect of IL-12 on CD40L expression was functionally relevant, we have studied cognate T cell interactions with B cells. We choose not to perform studies with macrophages, because IFN-γ induction by IL-12 would interfere strongly with the effect of CD40L on these cells. Optimal activation of B cells by Th cells requires direct contact between the two cell types through CD40 and CD40L (1). When we cultured tonsillar T (irradiated) and B cells together with anti-CD3 mAb and P815-CD80 cells, we found that addition of IL-12 clearly enhanced B cell proliferation and IgG production. An effect of IL-12 on B cells can be excluded because resting and activated B cells do not express IL-12R (53). Moreover, in control experiments in which we cultured B cells with 3T6 mouse fibroblasts transfected with CD40L and exogenous IL-4, IL-12 did not have any effect on B cell proliferation (data not shown). Thus, enhancement of B cell growth by IL-12 can be considered as proceeding entirely through effects on Th cell function. Besides cognate interactions, cytokines are also involved in T cell-dependent B cell activation. IL-12 strongly enhances IFN-γ production by T cells, but IFN-γ inhibits CD40L-induced B cell proliferation (54). IFN-γ is thus probably not involved in enhanced B cell proliferation as observed here. Moreover, we have added an excess of IL-4 to the T-B cell cultures for B cell proliferation to exclude indirect effects of IL-12 on T cell cytokine secretion. Finally, B cell proliferation was completely blocked by an mAb against CD40L. Similar data were also obtained for IgG production, which was enhanced by IL-12 and partially blocked by anti-CD40L. Therefore, we suggest that IL-12 has a helper effect on B cell function as a result of up-regulated CD40L expression on T cells.

IL-12 favors Th1 development and enhances IFN-γ production and CTL activity (20). Our findings now indicate that in vivo effects of IL-12 (26–31) on humoral immunity might result from up-regulating CD40L expression on T cells and their helper activity for B cell growth and Ab production. This effect of IL-12 on IL-12R-mediated effects might also modulate inflammatory processes, because CD40-CD40L interaction results in increased cytokine and gelatinase production by macrophages (12, 13, 17–19) and enhances antimicrobial defenses against intracellular organisms (14). Antileishmanial effects of IL-12 have been observed in IFN-γ knockout mice (25), and additional experiments should be performed to study whether the IFN-γ-independent effect is partly mediated by CD40L. Although the biologic significance of our findings for APC physiology requires further investigation in IFN-γ knockout mice or IFN-γR knockout mice, the induction of CD40L by IL-12 is probably part of an important regulatory circuit between APC and T cells. B7 and IL-12 both enhance CD40L expression on activated T cells and their IFN-γ production in a synergistic way (49). CD40L and IFN-γ, in turn, directly stimulate IL-12 production by macrophages and DC (17–19), and they enhance the expression of costimulatory molecules such as B7-1, B7-2, and intercellular adhesion molecule-1 on the APC (5, 15, 16). Finally, a signal delivered to T cells via CD40L triggering can up-regulate T cell activation and synergize with IL-12 to further induce IFN-γ production (55).

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References


