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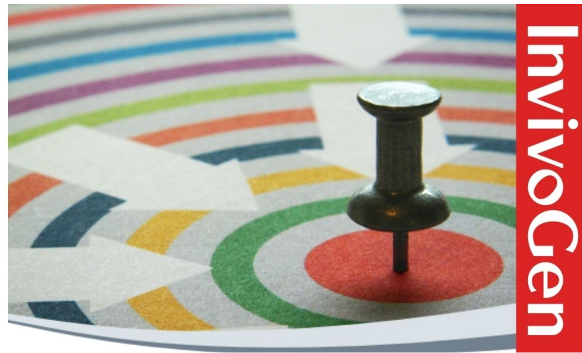

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REGULATION OF HUMAN LYMPHOCYTE PROLIFERATION BY A HETERODIMERIC CYTOKINE, IL-12 (CYTOTOXIC LYMPHOCYTE MATURATION FACTOR)

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IL-12 is a heterodimeric cytokine that was identified on the basis of its ability to synergize with IL-2 in the induction of cytotoxic effector cells and was originally called cytotoxic lymphocyte maturation factor (CLMF). IL-12 was also found to stimulate the proliferation of PHA-activated lymphoblasts which were >90% CD3+ T cells. In this report we further characterize the effects of IL-12 on lymphocyte proliferation. Studies with purified subpopulations of PHA-activated lymphoblasts and with cloned lines of human T cells indicated that IL-12 caused the proliferation of activated T cells of both the CD4+ and CD8+ subsets. This effect of IL-12 was independent of IL-2 because it was not blocked by antibodies to either IL-2 or IL-2R. The maximum proliferation induced by IL-12 was 31 to 72% of the maximum caused by IL-2; however, IL-12 was active at a lower effective concentration (EC_{50} = 8.5 ± 1.3 pM) than IL-2 (EC_{50} = 52 ± 8 pM). Combination of suboptimal amounts of IL-12 and IL-2 resulted in additive proliferation, up to the maximum induced by IL-2 alone. IL-12 also caused the proliferation of lymphocytes activated by culture with IL-2 for 6 to 12 days. CD56+ NK cells were among the IL-12-responsive cells in the IL-2-activated lymphocyte population. Unlike IL-2 or IL-7, IL-12 caused little or no proliferation of resting peripheral blood mononuclear cells (PBMC). In this regard, IL-12 was similar to IL-4. However, IL-12 could enhance the proliferation of resting PBMC caused by suboptimal amounts of IL-2, whereas IL-4 inhibited IL-2-induced PBMC proliferation. Thus, IL-12 is a growth factor for activated human T cells and NK cells; however, its spectrum of lymphocyte growth-promoting properties is distinct from that of IL-2, IL-4, or IL-7.

The proliferation of Ag-reactive lymphocytes is a central feature of the immune response. This proliferation is mediated via the interaction of cytokine growth factors with their receptors on the surfaces of activated lymphoid cells (1). IL-2 (2–4), IL-4 (5–7), and IL-7 (8, 9) have all been shown to be capable of stimulating the growth of activated human lymphocytes of various lineages. In addition, both IL-2 (10–12) and IL-7 (8, 9, 13) were shown to stimulate the proliferation of resting human PBL in the apparent absence of other mitogenic stimuli. IL-4, on the other hand, caused little or no proliferation of resting PBL, and inhibited IL-2-induced proliferation of resting PBL when added to cultures simultaneously with IL-2 (7, 14–16). IL-1 (17, 18), IL-6 (18), IL-9 (19), IL-10 (20), GM-CSF (21), and TNF (15, 22) have also been shown to act as stimulants or costimulants of lymphocyte proliferation, at least under some experimental conditions.

IL-12 (originally called CLMF[2]) is a heterodimeric lymphokine composed of disulfide-linked subunits with molecular masses of 40 and 35 kDa (23). The cDNA encoding this lymphokine have recently been cloned and expressed, and we proposed that the lymphokine be provisionally designated IL-12 (24). IL-12 was originally identified on the basis of its ability to synergize with suboptimal concentrations of IL-2 to facilitate cytotoxic lymphocyte responses (23, 25, 26), and was subsequently found to act as a growth factor for PHA-activated human T lymphoblasts (23).

NKSF is a heterodimeric cytokine closely related or identical to IL-12 (27, 28). NKSF was identified on the basis of its ability to induce the production of IFN-γ by resting human PBMC and was also shown to act as a costimulant of human lymphocyte proliferation in combination with PHA or a phorbol ester (27). In this report, we characterize more fully the effects of IL-12 on the proliferation of both resting and activated human PBMC and demonstrate that its spectrum of lymphocyte growth-promoting properties is distinct from that of IL-2, IL-4, or IL-7.

MATERIALS AND METHODS

Media and reagents . TCM was a 1:1 mixture of RPMI 1640 and Dulbecco's modified Eagle's medium, supplemented with 0.1 mM nonessential amino acids, 60 μg/ml arginine HCl, 10 mM HEPES buffer, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (all available from GIBCO, Grand Island, NY), 50 μM 2-ME (Fisher Scientific, Fair Lawn, NJ), 1 mg/ml dextrose (Fisher), and 5% human AB serum (Irvine Scientific, Santa Ana, CA). PHA-P was

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[2] Abbreviations used in this paper: CLMF, cytotoxic lymphocyte maturation factor; EC_{50}, 50% effective concentration; LAK, lymphokine-activated killer; NKSF, natural killer cell stimulatory factor; PE, phycoerythrin; TCM, tissue culture medium.

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from Difco Laboratories, Detroit, MI. Human rIL-2 (>95% pure as analyzed by SDS-PAGE) was supplied by Dr. F. Khan, Department of Bioprocess Development, Hoffmann-La Roche Inc. rIL-4 was from Genzyme, Boston, MA, or from Amgen, Thousand Oaks, CA; both preparations gave equivalent results in our assays. rIL-7 was from Pepro Tech, Inc., Rocky Hill, NJ. mAb 2A3 against the human IL-2R p55 protein (29) was provided by Dr. J. Hakimi, Department of Immunopharmacology, Hoffmann-La Roche Inc. mAb Mik-β1 against the human IL-2R p75 protein (30) was a gift from Dr. M. Tsudo, Internal Medicine Department, Unichika Central Hospital, Kyoto-fu, Japan.

IL-12 and antibodies to IL-12. Human IL-12 was produced by activation of NC-37 B lymphoblastoid cells with PMA plus calcium ionophore A23187 (Sigma) and purified as described in Stern et al. (23). The NC-37-derived IL-12 used in these studies was >95% pure as assessed by SDS-PAGE. rIL-12 was produced by cotransfection of COS cells with a 1:1 molar ratio of the two subunit cDNAs of IL-12 as described in Gubler et al. (24). Crude supernatant fluid from cultures of doubly transfected cells was used as a source of rCLMF in these experiments. Such supernatant fluid was found to contain free 40-kDa subunit as well as heterodimeric IL-12; however, the free 40-kDa subunit does not display significant biologic activity in lymphocyte proliferation or LAK cell induction assays and does not appear to bind to the IL-12R (24) (R. Chizzonite, unpublished results). Likewise, supernatant fluids from cultures of COS cells transfected with either IL-12 subunit cDNA alone displayed no activity in lymphocyte proliferation or LAK cell induction assays (24). Rat anti-human IL-12 mAb 4A1 was isolated and purified as previously described (23).[3] This antibody is specific for the 40 kDa subunit of IL-12, and its isotype is IgG2b.

Lymphocyte proliferation assays. PHA-activated human peripheral blood lymphoblasts were produced as previously described (23). IL-2-activated lymphoblasts were generated by culturing human PBMC, 1 × 10^6/ml, in TCM containing 5% AB serum and 100–200 U/ml rIL-2. The cultures were supplemented on day 5 with an additional 100 U/ml IL-2 and harvested for assay on days 6 to 12, as indicated in *Results*. The cytokine-induced proliferation of human PHA- or IL-2-activated peripheral blood lymphoblasts or of human T cell lines was assessed in 48-h assays as previously described (23). To evaluate the response of resting PBMC to various lymphokines, PBMC were isolated (31) from normal donors and suspended in TCM at a concentration of 2 × 10^6 cells/ml. Aliquots of 50 μl of the cell suspension were mixed in the wells of Costar 3596 microplates with 25-μl aliquots of cytokine-containing solutions in a total volume of 100 μl. The cultures were incubated at 37°C for the indicated number of days. Approximately 16 to 18 h before harvest, 50 μl of [^{3}H]thymidine (New England Nuclear), 10 μCi/ml in TCM, was added to each well. The culture contents were harvested onto glass fiber filter mats by means of a cell harvester (Tomtec, Orange, CT), and [^{3}H]thymidine incorporation into cellular DNA was measured by liquid scintillation spectroscopy. All samples were assayed in triplicate.

IL-2-dependent human T cell lines. IL-2-dependent human T cell lines were isolated and maintained as previously described (32, 33). LG37 and HK.L41 are malaria peptide-specific $CD4^+8^-$ T cell lines whereas MF15 is an alloreactive $CD4^-8^+$ T cell line.

Cell separation studies. T cell subpopulations and T cell-depleted, NK-enriched lymphocyte populations were prepared by panning using negative selection. PHA- or IL-2-activated lymphocyte populations (25 × 10^6 cells/ml) were treated with mouse monoclonal anti-human CD4 at 10 μg/ml, anti-human CD8 at 15 μg/ml, (both from PharMingen, San Diego, CA), or anti-human CD3 at 15 μg/ml (Ortho, Raritan, NJ) for 20 min at room temperature. The cells were then washed and panned on plates coated with affinity-purified goat anti-mouse IgG (Tago, Burlingame, CA) as described by Wysocki and Sato (34). The nonadherent cells recovered after panning were washed and evaluated in lymphocyte proliferation assays. Alternatively, in some experiments lymphocyte populations depleted of $CD3^+$ cells by panning were further treated with anti-CD3 and C (31) before assay. The purity of all cell populations was assessed by immunofluorescent staining as described below.

Immunofluorescent staining. FITC-conjugated mouse mAb to human CD3, CD4, CD8, and CD20, PE-conjugated mouse monoclonal antibodies to human CD14, CD25, and CD56, and control FITC- or PE-conjugated mouse IgG were from Becton-Dickinson, Mountain View, CA. FITC-conjugated goat anti-mouse IgG was from Ortho. Aliquots of 10 μl of FITC- or PE-conjugated mAb or control IgG were added to 0.1-ml aliquots of mononuclear cell suspensions (10^7 cells/ml in Dulbecco's PBS (GIBCO) with 2% heat-inactivated FCS and 0.1% sodium azide). The mixtures were incubated for 30 min on ice, and the cells were then washed and analyzed by flow cytometry using a FACSCAN (Becton Dickinson).

RESULTS

Effects of IL-12 on activated PBMC. We previously reported that IL-12 caused the proliferation of PHA-activated human lymphoblasts and that this activity of IL-12 was blocked by mAb to IL-12 but not by a potent neutralizing antibody to IL-2 (23, 24). In the present study, IL-12-induced proliferation of human PHA blasts was also found to be unaffected by the addition of neutralizing antibodies to the p55 and p75 components of the IL-2R at concentrations which caused complete abrogation of IL-2-induced proliferation (Fig. 1). Hence, IL-12-induced proliferation of human PHA blasts was clearly independent of both IL-2 and the IL-2R. Further experiments were undertaken to characterize this activity of IL-12.

PHA-activated lymphoblast populations in our experiments routinely consisted of greater than 90% $CD3^+$ T cells. We therefore compared the growth-promoting activity of IL-12 on human PHA blasts to that of three previously identified T cell growth factors—IL-2, IL-4, and IL-7. The responsiveness of human PHA blasts to IL-12 was relatively transient. Peak responses were observed on day 4 after activation, but by day 6 after activation, PHA blasts displayed little or no proliferative response to IL-12 (data not shown). Peak proliferative responses of human PHA blasts to IL-2 were also observed on day 4 after activation; however, in contrast to IL-12, IL-2 typically elicited substantial proliferative responses by 6-day PHA blasts. All comparative studies were performed on PHA blasts 4 days after activation. Eight such experiments were performed, and the results of a representative experiment are shown in Figure 2*A*. The maximum proliferation induced by IL-12 was similar to the maximum caused by IL-4 or IL-7 but less than the maximum prolif-

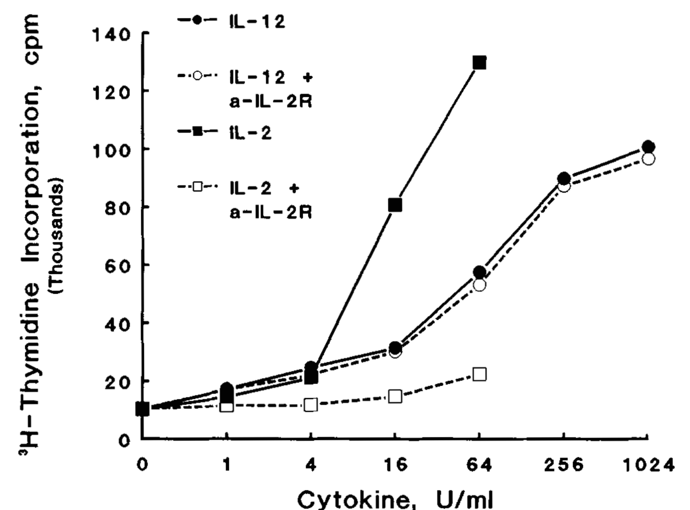

Figure 1. mAb to IL-2R inhibit IL-2- but not IL-12-induced proliferation of PHA-activated human lymphoblasts. Human PHA-activated lymphoblasts were incubated for 48 h in the indicated concentrations of rIL-2 (*squares*) or purified, NC-37-derived IL-12 (*circles*) in the presence (*open symbols*) or absence (*closed symbols*) of a mixture of monoclonal 2A3 anti-human IL-2R p55 and monoclonal Mik-β1 anti-human IL-2R p75 (*a-IL-2R*). The concentration of each mAb in the assay was 10 μg/ml. [^{3}H]thymidine was added 18 h before the end of the assay.

[3] Chizzonite, R. C., T. Truitt, F. J. Podlaski, A. G. Wolitzky, P. M. Quinn, P. Nunes, A. S. Stern, and M. K. Gately. IL-12 monoclonal antibodies specific for the 40 kDa subunit block receptor binding and biological activity on activated human lymphoblasts. *Submitted for publication.*

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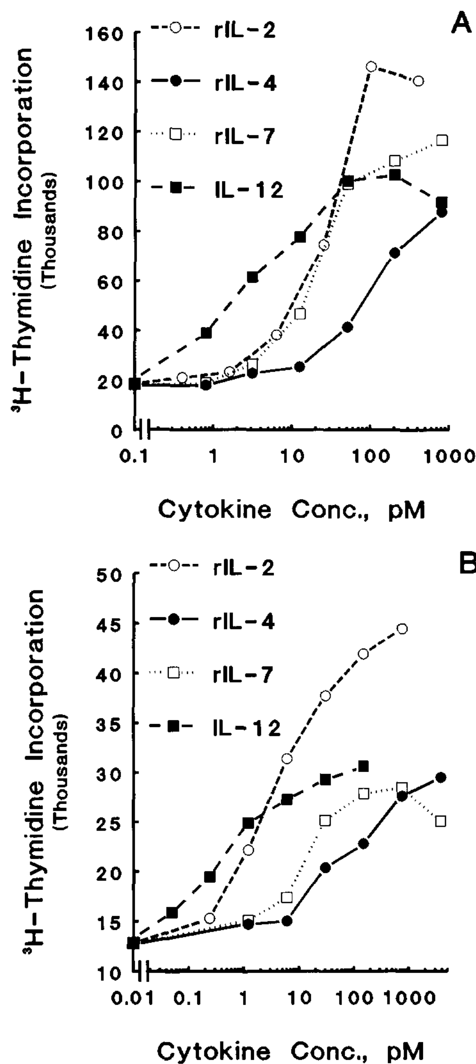

Figure 2. Dose-response curves for the proliferation-inducing activities of human rIL-2, rIL-4, rIL-7, and IL-12 on human PHA-activated lymphoblasts (*A*) and IL-2-activated lymphoblasts (*B*). The proliferation of 4-day PHA- and 7-day IL-2-activated lymphoblasts was assessed in 48 hr assays as previously described (23). One pM purified, NC-37-derived IL-12 is equal to 6 units/ml. The results are representative of eight separate experiments performed with each type of lymphoblast.

eration induced by IL-2. In eight experiments the ratio of the maximum PHA blast proliferation caused by IL-12, IL-4, or IL-7 to the maximum proliferation caused by IL-2 was 0.50 ± 0.05, 0.55 ± 0.03, and 0.70 ± 0.07, respectively. Of these four growth-promoting lymphokines, IL-12 was active at the lowest concentration. The concentrations of IL-12, IL-2, IL-4, and IL-7 required to stimulate half-maximum proliferation of 4-day human PHA blasts were 8.5 ± 1.3 pM, 52 ± 8 pM, 189 ± 40 pM, and 80 ± 20 pM, respectively. We previously showed that IL-12 could synergize with suboptimal concentrations of IL-2 in causing the induction of LAK cells in the presence of hydrocortisone (23, 24). However, when suboptimal concentrations of IL-2 and IL-12 were added together to the PHA blast proliferation assay, no clear synergy was observed (Table I). Rather, mixtures of IL-2 and IL-12 caused proliferation which was approximately additive, up to the maximum proliferation induced by optimal concentrations of IL-2 alone.

In view of the observation that the maximum PHA blast proliferation induced by IL-12 was about half that caused

TABLE I

Effects of purified human IL-12 and rIL-2 on the proliferation of human PHA-activated lymphoblasts[a]

Cytokine Added:		[³H]Thymidine Incorporated by PHA-activated Lymphoblasts (mean cpm ± 1 S.E.M.)
Human IL-12 (U/ml)	Human rIL-2 (U/ml)	
0	0	9,976 ± 374
200	0	60,980 ± 1,713
50	0	38,817 ± 884
12.5	0	21,268 ± 2,132
3.1	0	13,648 ± 731
0	64	89,894 ± 6,835
0	16	79,789 ± 5,835
0	4	21,029 ± 1,145
0	1	10,989 ± 898
50	16	88,736 ± 2,879
12.5	16	93,162 ± 3,166
3.1	16	92,140 ± 6,086
50	4	62,050 ± 2,408
12.5	4	40,628 ± 2,196
3.1	4	31,144 ± 3,754
50	1	41,445 ± 4,133
12.5	1	30,038 ± 853
3.1	1	20,812 ± 994

[a] PHA-activated human peripheral blood lymphoblasts from 4-day cultures were tested for their ability to proliferate in response to purified, NC-37-derived human IL-12 and/or human rIL-2 as previously described (23).

by IL-2, it seemed possible that only a subset of the PHA-induced lymphoblast population might be responsive to IL-12. However, despite a 2- to 3-fold increase in viable cell number over the course of the 48-h proliferation assay, no significant changes were observed in the percentages of CD4⁺ T cells, CD8⁺ T cells, and CD20⁺ B cells, which together accounted for >98% of the cells within the PHA-activated lymphoblast populations (data not shown). Six experiments were performed in which cell populations enriched for CD4⁺ or CD8⁺ T cells were obtained by panning of 4-day PHA blasts and were tested for their ability to proliferate in response to IL-12. The results suggested that both CD4⁺ and CD8⁺ T cells, when appropriately activated, could proliferate in response to IL-12 (Fig. 3). This conclusion was confirmed by the results of studies in which the effects of rIL-12 on cloned, IL-2-dependent lines of human T cells were studied. Six CD4⁺ T cell lines and one CD8⁺ line were tested. Two CD4⁺ lines and the CD8⁺ line displayed dose-dependent proliferative responses to COS cell-derived rIL-12 but did not respond to control fluid from cultures of mock-transfected COS cells (Fig. 4). The IL-2-dependent human T cell lines used in these studies require periodic restimulation with PHA and irradiated PBMC for long-term growth (32, 33) and were responsive to IL-12 for a relatively brief interval of time following restimulation. Optimal responsiveness occurred between days 6 and 8 after restimulation and waned thereafter (data not shown). The results with these T cell lines confirm the conclusion that IL-12 can cause the proliferation of activated T cells of both the CD4⁺ and CD8⁺ subsets.

Human PBMC activated by agents other than PHA were also tested for their ability to proliferate in response to IL-12. IL-12 caused dose-dependent proliferation of PBMC activated by culture with concanavalin A, pokeweed mitogen, phorbol ester plus calcium ionophore, immobilized anti-CD3, or irradiated allogeneic PBMC (data not shown). In addition, culture of PBMC in the presence of 100 to 200 U/ml of IL-2 without any other mitogenic agent also resulted in the generation of a population of

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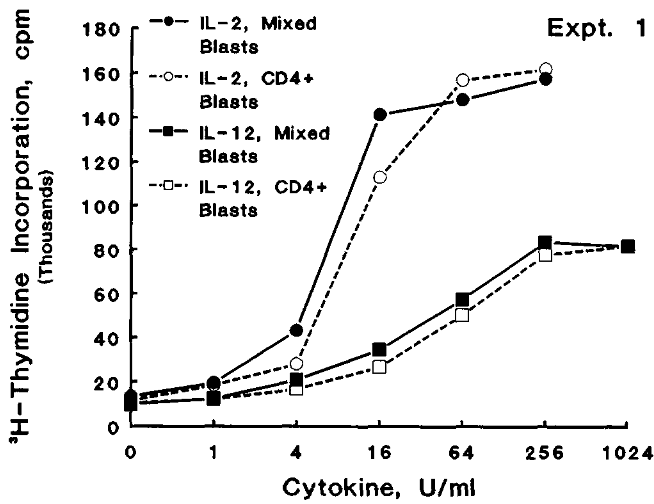


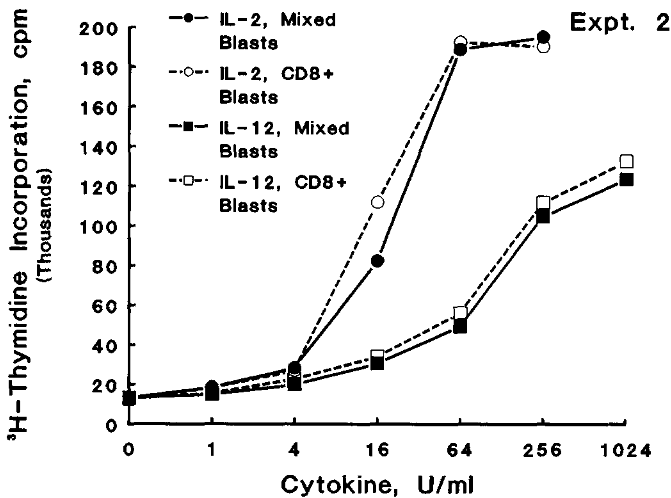
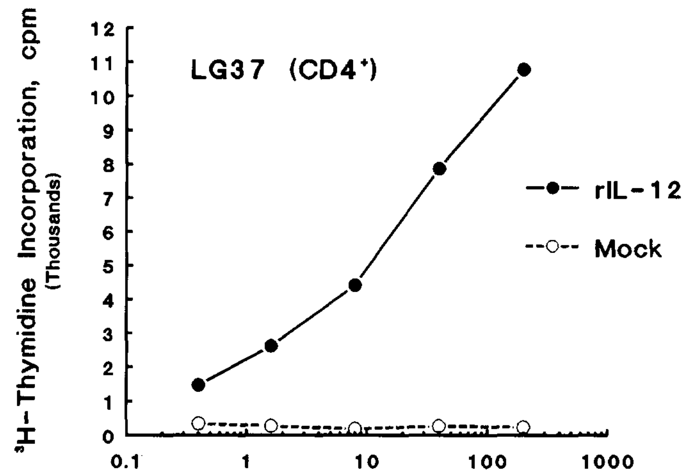
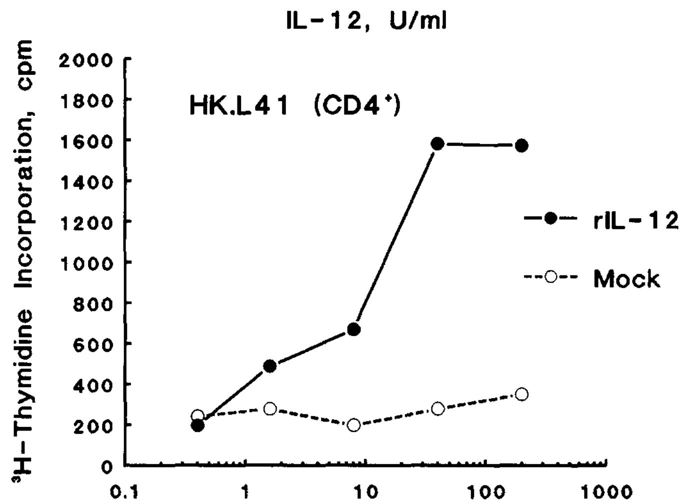


Figure 3. Both CD4⁺ and CD8⁺ PHA-activated human lymphoblasts proliferate in response to IL-12. Enriched populations of CD4⁺ and CD8⁺ T cells were obtained by panning using negative selection and tested for their ability to proliferate in response to rIL-2 and to purified, NC-37-derived IL-12. In experiment 1, the unseparated PHA blasts contained 57% CD4⁺ cells and 35% CD8⁺ cells. After panning, the CD4⁺ T cell-enriched population was composed of 93% CD4⁺ cells and 3% residual CD8⁺ cells as detected by staining with anti-CD8 followed by FITC-conjugated anti-mouse IgG. In experiment 2, the percentages of CD4⁺ and CD8⁺ cells in the unseparated PHA blast population were 32% and 61%, respectively. The CD8⁺ T cell-enriched population contained 91% CD8⁺ cells and 4% residual CD4⁺ cells. All cell populations contained 2 to 6% CD20⁺ B cells. Less than 1% of the cells stained with anti-CD56 or anti-CD14. These results are representative of six separate experiments.

lymphocytes responsive to IL-12 (Fig. 2*B*). Peak responsiveness of IL-2-activated PBMC occurred after 7 to 8 days of culture, and IL-2-activated lymphoblasts continued to be responsive to IL-12 at least through day 12 of culture (data not shown). In eight experiments in which the proliferative responses of IL-2-activated lymphoblasts to IL-12, IL-2, IL-4, and IL-7 were compared, IL-2 was again observed to induce the highest levels of proliferation (Fig. 2*B*). The ratio of the maximum proliferation induced by IL-12, IL-4, or IL-7 to that induced by IL-2 was 0.42 ± 0.04, 0.33 ± 0.06, and 0.26 ± 0.06, respectively. The concentrations of IL-12, IL-2, IL-4, and IL-7 that were required to stimulate half-maximum proliferation of 7-day IL-2 blasts were 1.4 ± 0.3 pM, 10.5 ± 1.7 pM, 50 ± 5 pM, and 32 ± 5 pM, respectively.

Phenotyping of the IL-2-activated lymphoblast populations indicated that in addition to CD3⁺ T cells, these cell populations contained 30–70% CD56⁺ NK cells, in





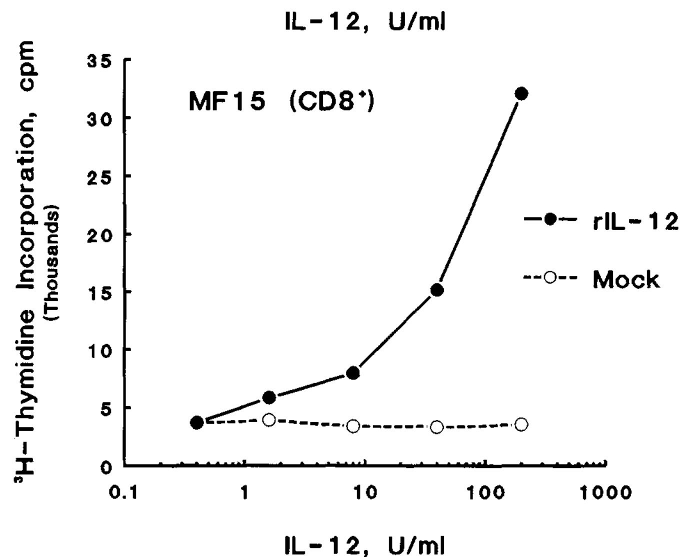


Figure 4. Proliferation of CD4⁺ and CD8⁺ human T cell lines in response to IL-12. The proliferation of human T cell lines LG37 and HK.L41 (both CD4⁺) and MF15 (CD8⁺) was measured in 48-h assays as described in Stern et al. (23). The cell lines were cultured either with the indicated amounts of rIL-12 contained in supernatant fluid from cultures of COS cells transfected with both IL-12 subunit cDNAs (*closed circles*) or with equal volumes of supernatant fluid from cultures of mock transfected COS cells (*open circles*).

agreement with a recent report that CD56⁺ NK cells constitute the major subset of resting PBMC that proliferates in response to IL-2 (12). To determine whether the IL-2-

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activated $CD56^+$ cells could proliferate in response to IL-12, we removed $CD3^+$ cells from the IL-2-activated PBMC populations by a combination of panning and complement depletion techniques. The resulting cell populations, which contained ≥90% $CD56^+$ cells and ≤5% $CD3^+$ cells, proliferated in response to IL-12 similar to the unseparated cell populations from which they were derived (Fig. 5). These results indicate that IL-12 can act as a growth factor for activated NK cells, as well as for activated T cells.

Effects of IL-12 on resting PBMC. In contrast to its ability to cause the proliferation of activated T and NK cells at concentrations <10 pM, IL-12, at concentrations as high as 200 pM, did not cause significant proliferation of resting PBMC in a 6-day assay (Table II). In this regard, IL-12 was similar to IL-4 but unlike IL-2 or IL-7, both of which caused significant proliferation of resting PBMC in agreement with previously published results (8–13). Several investigators have observed that IL-4 can inhibit IL-2-induced proliferation of resting PBMC when added to the cultures simultaneously with IL-2 (7, 14–16). Experiments were performed to determine whether IL-12 would also display this property. However, in contrast to the inhibitory effect of IL-4, IL-12 enhanced IL-2-induced proliferation of resting PBMC in cultures which received a suboptimal concentration (1–25 U/ml) of IL-2 (Fig. 6). That this effect was, in fact, mediated by IL-12 was demonstrated by the ability of a neutralizing monoclonal rat anti-human IL-12 antibody to inhibit IL-12-mediated enhancement of IL-2-induced PBMC proliferation (Table III). Likewise, rIL-12, as well as natural IL-12, was shown to enhance the proliferation of resting PBMC caused by suboptimal concentrations of IL-2 (data not shown). On

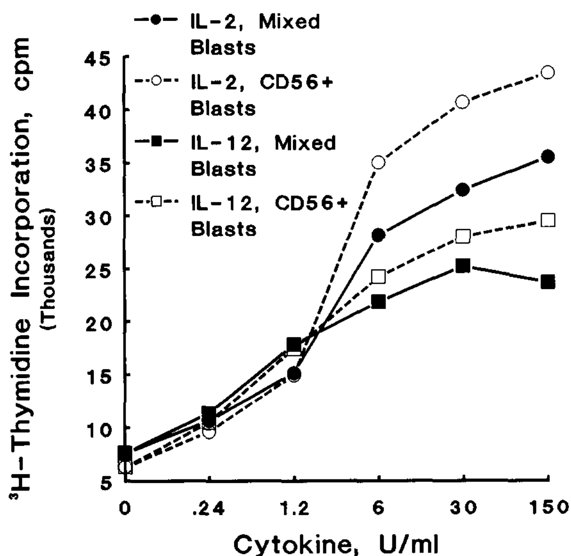

Figure 5. $CD56^+$ IL-2-activated lymphoblasts proliferate in response to IL-12. Human PBMC were cultured with rIL-2 for 8 days, and an enriched population of $CD3^-56^+$ NK cells was prepared by removal of $CD3^+$ cells by treatment with anti-CD3 and panning followed by treatment with anti-CD3 and C. The unseparated IL-2-activated PBMC contained 60% $CD3^-56^+$ cells, 7% $CD3^+56^+$ cells, and 26% $CD3^+56^-$ cells. Following depletion of $CD3^+$ cells, the $CD56^+$ cell-enriched population contained 93% $CD3^-56^+$ cells, 4% $CD3^+56^+$ cells, and <1% $CD3^+56^-$ cells. Both cell populations contained ≤1% $CD14^+$ monocytes and <1% $CD20^+$ B cells. The unseparated cell population (*closed symbols*) and the cell population enriched in $CD56^+$ NK cells (*open symbols*) were tested for their ability to proliferate in response to human rIL-2 (*circles*) or purified NC-37-derived IL-12 (*squares*) in a 48-h assay as previously described elsewhere (23). These results are representative of four separate experiments.

TABLE II

Human rIL-2 and rIL-7, but not rIL-4 or IL-12, stimulate the proliferation of resting human PBMC[a]

Cytokine	Concentration (pM)	[³H]Thymidine Incorporation (mean cpm ± 1 S.E.M.)
None		129 ± 6
rIL-2	200	18,234 ± 2175
rIL-2	50	7,214 ± 710
rIL-2	12.5	2,028 ± 265
rIL-2	3.1	477 ± 124
rIL-4	200	156 ± 8
rIL-4	50	253 ± 104
rIL-4	12.5	589 ± 89
rIL-4	3.1	137 ± 37
rIL-7	200	9,120 ± 494
rIL-7	50	1,556 ± 503
rIL-7	12.5	179 ± 28
rIL-7	3.1	236 ± 19
IL-12	200	330 ± 195
IL-12	50	254 ± 16
IL-12	12.5	269 ± 75
IL-12	3.1	77 ± 13

[a] Human PBMC were incubated for 6 days at 37°C with the indicated amounts of rIL-2, rIL-4, rIL-7 or purified, NC-37-derived IL-12. [³H]Thymidine was added to the cultures 18 h before the end of the incubation.

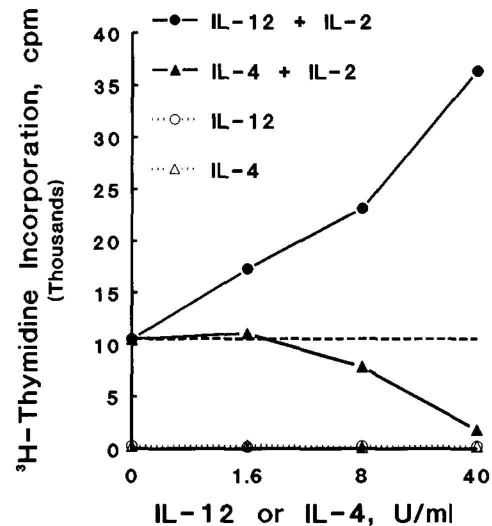

Figure 6. IL-12 enhances and IL-4 inhibits IL-2-induced proliferation of resting PBMC. Human PBMC were cultured for 7 days in the indicated amounts of purified NC-37-derived IL-12 (*circles*) or human rIL-4 (*triangles*) with (*closed symbols*) or without (*open symbols*) human rIL-2, 5 U/ml. [³H]Thymidine was added 18 h before the end of culture. The *dashed line* indicates the level of [³H]thymidine incorporation by PBMC incubated in 5 U/ml rIL-2 without rIL-4 or IL-12.

the other hand, in cultures of resting PBMC which received optimal concentrations of IL-2 (100 to 500 U/ml), IL-12 either had no effect or caused modest (≤35%) inhibition of proliferation (data not shown).

Kinetic studies were performed to determine whether IL-12-mediated enhancement of IL-2-induced PBMC proliferation represented an actual increase in the peak proliferative response or a shift in the kinetics of the response. The results from each of two experiments (Fig. 7) indicated that for a given concentration of IL-2, IL-12 both increased the peak proliferation and prolonged the proliferative response so that the peak in the presence of IL-12 typically occurred later. Interestingly, the results of adding IL-12 to cultures containing a given concentration of IL-2 were similar to the results of increasing the concentration of IL-2 in the absence of IL-12, in terms of enhancing both the magnitude and the duration of the proliferative response. For example, the responses in-

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TABLE III

Neutralizing antibody to IL-12 inhibits IL-12-mediated enhancement of IL-2-induced proliferation of resting human PBMC[a]

Cytokines Added:		[^{3}H]Thymidine Incorporated (mean ± 1 S.E.M.) in presence of:		
rIL-2 (U/ml)	IL-12 (U/ml)	No added antibody	4A1 Anti-IL-12 IgG[b]	Normal rat IgG[b]
0	0	897 ± 577		
0	5	362 ± 86		
0	25	449 ± 79		
0	125	1,160 ± 268		
5	0	820 ± 137	301 ± 50	677 ± 27
5	5	1,969 ± 327	265 ± 105	2,046 ± 449
5	25	5,359 ± 1,188	665 ± 101	6,101 ± 570
5	125	14,404 ± 1,792	1,163 ± 375	12,647 ± 3,340
25	0	13,832 ± 1,764	12,774 ± 1,096	15,880 ± 2,491
25	5	32,521 ± 3,990	12,805 ± 2,841	22,097 ± 3,909
25	25	40,217 ± 5,952	12,028 ± 2,960	36,711 ± 3,079
25	125	57,428 ± 1,757	24,030 ± 2,061	57,297 ± 3,397

[a] Human PBMC were incubated for 9 days at 37°C with the indicated cytokines and IgG. [^{3}H]Thymidine was added 18 h before the end of culture. The results are representative of 2 separate experiments.

[b] The concentration of monoclonal 4A1 anti-IL-12 IgG or normal rat IgG (Sigma) was 10 μg/ml.

duced by 125 U/ml IL-2 were comparable to those induced by 25 U/ml IL-2 + 125 U/ml IL-12 (Fig. 7). Hence the effect of IL-12 appeared to be that of reducing the concentration of IL-2 which was required to elicit a proliferative response of a given magnitude and duration.

To gain further insight into the mechanism by which IL-12 enhances IL-2-dependent proliferation of resting PBMC, flow cytometry was used to examine the expression of IL-2R p55 protein (CD25) and the subset distribution of PBMC cultured with IL-2 or IL-2 + IL-12 (Table IV). In the presence of either IL-2 or IL-2 + IL-12, the percentage of $CD3^-56^+$ NK cells increased gradually until the time at which DNA synthesis peaked. As DNA synthesis declined, the lymphocyte subset distribution remained relatively constant. These results suggest that $CD56^+$ NK cells constituted the predominant population of proliferating cells in these cultures. IL-12 by itself caused little or no enhancement of CD25 expression either at day 3 (Table IV) or after culture for 6 to 8 days (data not shown). Likewise, the initial IL-2-induced upregulation of CD25 expression was not appreciably enhanced by the presence of IL-12. However, in each of four separate experiments the decline in DNA synthesis in cultures containing suboptimal amounts of IL-2 without IL-12 was heralded by a fall in the fluorescence intensity of $CD25^+$ cells whereas in cultures containing an identical amount of IL-2 plus IL-12, the expression of CD25 was maintained at maximum levels for an additional 1–2 days. Hence, IL-12 appeared to prolong the IL-2-induced expression of IL-2R p55 protein, and this may have contributed to the increased proliferation observed in cultures containing IL-12 plus a suboptimal amount of IL-2. However, late in culture (e.g., on day 9 in the experiment shown in Table IV) levels of DNA synthesis did not correlate with CD25 expression. Levels of DNA synthesis in cultures containing IL-12 + IL-2 were greater than would have been expected based on the levels of CD25 expression. One possible explanation for this observation is that proliferation at late times in such cultures was driven primarily by IL-12 rather than by IL-2. Alternatively, IL-2 could have been driving proliferation via the intermediate affinity IL-2R.

Although IL-12 alone did not induce significant proliferation of resting PBMC or increased expression of CD25, IL-12 by itself did enhance the expression of CD56, as reflected by an increase in the relative fluorescence intensity of $CD3^-56^+$ cells (Table IV). This is consistent with the observation that culture of PBMC with IL-12 alone results in enhanced lytic activity of NK cells (27) (M. K. Gately, unpublished results).

DISCUSSION

IL-12 is a heterodimeric lymphokine which was identified on the basis of its ability to synergize with IL-2 in facilitating cytotoxic lymphocyte responses (23, 26) and was also shown to cause the proliferation of PHA-activated peripheral blood lymphoblasts (23). In this report, we have further characterized the effects of IL-12 in regulating the proliferation of resting and activated PBMC. Experiments with PBMC activated by mitogens or by IL-2 demonstrated that IL-12 is a growth factor for both activated T cells and NK cells. For both PHA-activated lymphoblasts and IL-2-activated lymphoblasts, the magnitude of the maximum proliferative response elicited by IL-12 was similar to that elicited by IL-4 or IL-7 but less than that elicited by IL-2. In addition, the ability of PHA-activated lymphoblasts to proliferate in response to IL-12 appeared to be more transient than their ability to respond to IL-2. This observation is similar to the results of Spits et al. (5) who reported that the capacity of activated T cell clones to proliferate in response to IL-4 was more short-lived than their ability to respond to IL-2. Nevertheless, despite the similarity in the properties of IL-4 and IL-12 as growth factors for activated T cells, neutralizing monoclonal antibodies to human IL-12 did not inhibit IL-4-induced proliferation of PHA blasts, and a neutralizing polyclonal antibody to human IL-4 did not inhibit IL-12-induced PHA blast proliferation (M. K. Gately, unpublished results), suggesting that each of these two lymphokines exerts its proliferative effects independent of the other. In addition, the inability of neutralizing antibodies to IL-2 or the IL-2R to inhibit IL-12-induced proliferation of PHA blasts clearly indicates that IL-12 can act as a T cell growth factor independent of IL-2. With respect to the concentration of cytokine required to induce a proliferative response, IL-12 was the most potent of the four growth factors examined in these experiments, both on PHA blasts and on IL-2 blasts. The concentration of IL-12 required to induce half-maximum proliferation of either PHA blasts or IL-2 blasts was approximately 6-fold lower than the concentration of IL-2 needed for a half-maximum response and 9- to 35-fold lower than the EC_{50}s for IL-4 or IL-7.

Experiments with subpopulations of PHA-activated lymphoblasts enriched in $CD4^+$ T cells or in $CD8^+$ T cells suggested that activated lymphocytes from both T cell subsets are capable of proliferating in response to IL-12. This conclusion was confirmed by studies using cloned lines of $CD4^+$ or $CD8^+$ T cells. It is unclear why four of the six $CD4^+$ T cell lines which were tested failed to respond to IL-12. It is possible that different cell lines modulate their IL-12R differently and that the four unresponsive T cell lines might have been in a state of receptor down-modulation at the time of assay. Alternatively, they may have lost their capacity to become responsive to IL-12 during the course of long term culture,

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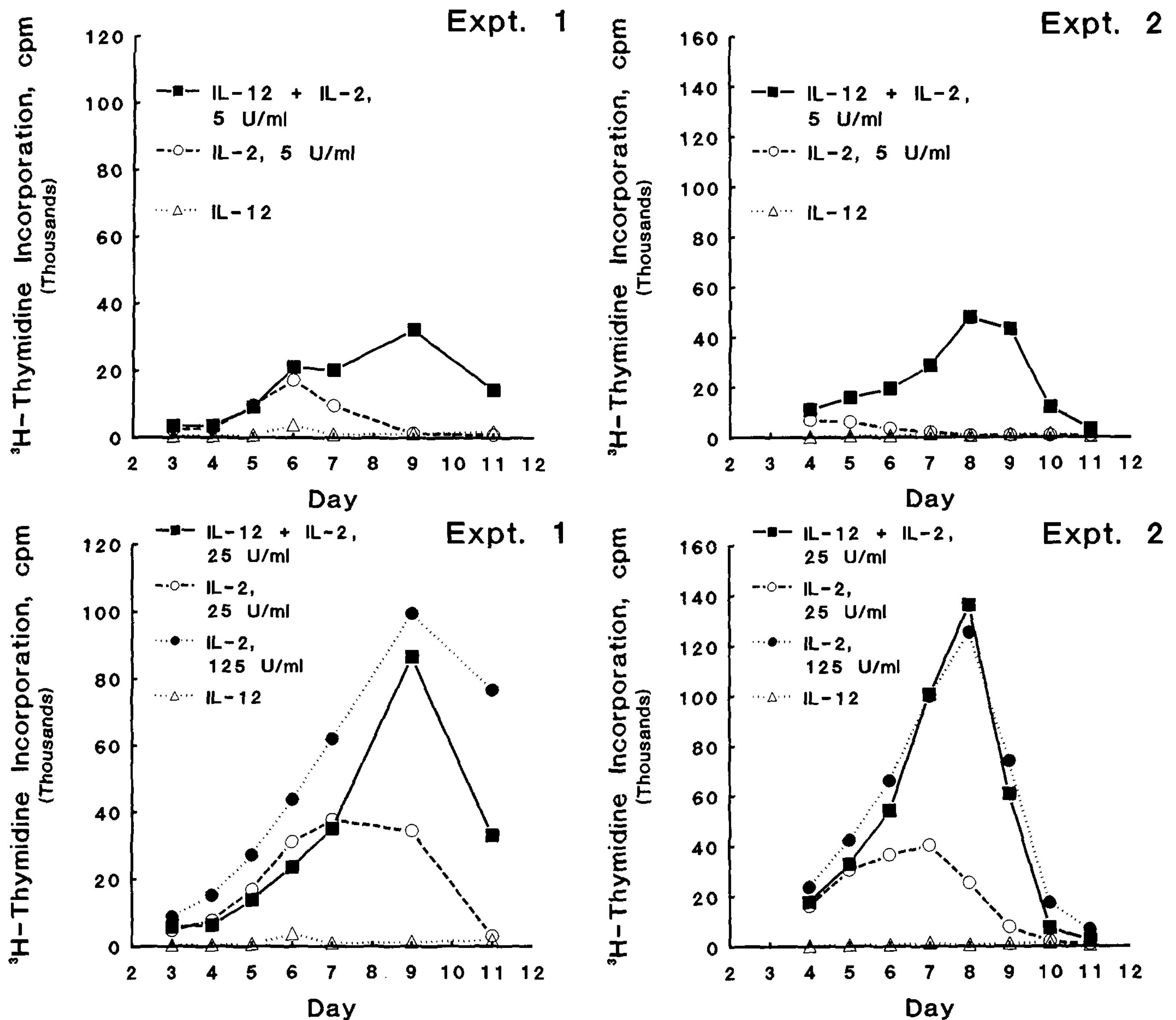

Figure 7. Kinetics of the proliferative response of resting human PBMC to rIL-2 with or without IL-12. Human PMBC were cultured for the indicated number of days with purified NC-37-derived IL-12, 125 U/ml, and/or human rIL-2 at the indicated concentrations. [^{3}H]Thymidine was added to each culture 18 h before harvest.

or there may exist IL-12-responsive and IL-12-unresponsive subsets of CD4$^+$ T cells. Further experiments are required to distinguish between these possibilities as well as to determine the frequency with which CD8$^+$ T cell lines respond to IL-12. Experiments with IL-2-activated lymphoblasts which had been depleted of CD3$^+$ cells strongly suggested that activated CD3$^-$56$^+$ NK cells are also capable of proliferating in response to IL-12. The ability of other subsets among the IL-2-activated lymphocyte populations (principally CD3$^+$56$^-$ and CD3$^+$56$^+$ cells) to proliferate in response to IL-12 was not examined in these studies. Likewise, the question of whether IL-12 can stimulate the proliferation of activated B cells was not addressed but is the subject of ongoing experiments.

Although IL-12, unlike IL-2 or IL-7, was not by itself significantly mitogenic for resting PBMC over the range of concentrations tested (1 to 200 pM), it did cause a dose-dependent enhancement of the proliferation induced by suboptimal concentrations of IL-2. That this effect was, in fact, mediated by IL-12 was indicated by the observations that both natural and rIL-12 displayed this activity and that a neutralizing mAb to IL-12 could inhibit this response. IL-12 appeared both to enhance and to prolong the proliferative response of resting PBMC to a suboptimal concentration of IL-2, similar to the changes which resulted from adding a more nearly optimal concentration of IL-2 without IL-12 (Fig. 7). There are several mechanisms by which this could occur. IL-12 may have altered the responding PBMC so as to enable them to proliferate in response to lower concentrations of IL-2. This might have resulted from an IL-12-mediated enhancement of IL-2R expression or from an alteration in the signal transduction process so that fewer occupied IL-2R were required to trigger a proliferative response. IL-12 by itself does not appear to up-regulate IL-2R expression (Table IV) (our unpublished data). However, the ability of IL-12 to prolong the IL-2-induced expression of IL-2R p55 is compatible with the hypothesis that enhanced prolifera-

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TABLE IV
Phenotypic analysis of human PBMC cultured in IL-2 with or without IL-12[a]

Cytokine (U/ml):		Phenotype (%)				[³H]Thymidine Incorporation[b]
rIL-2	IL-12	CD25⁺	CD3⁺56⁻	CD3⁺56⁺	CD3⁻56⁺	
Day 3:						
0	0	9 (407[c])	74	3	12 (465[d])	115
0	125	14 (445)	67	5	17 (623)	1,690
5	0	25 (555)	68	4	18 (598)	11,792
5	125	29 (582)	72	5	13 (622)	12,328
25	0	25 (585)	72	5	14 (611)	14,051
Day 6:						
5	0	35 (457)	59	5	28 (624)	22,918
5	125	56 (581)	46	9	38 (711)	38,188
25	0	72 (552)	35	9	51 (665)	78,983
Day 7:						
5	0	35 (438)	55	5	32 (624)	12,782
5	125	60 (510)	31	8	54 (699)	76,804
25	0	76 (476)	28	9	58 (653)	106,604
Day 8:						
5	0	25 (446)	61	5	25 (579)	7,607
5	125	44 (450)	23	7	66 (664)	74,658
25	0	54 (414)	26	8	61 (612)	54,884
Day 9:						
5	0	20 (433)	61	7	24 (568)	1,300
5	125	25 (409)	17	7	73 (654)	46,934
25	0	23 (397)	23	10	62 (604)	17,594

[a] Proliferation of resting human PBMC cultured in IL-2 ± IL-12 for the indicated number of days was measured in microcultures as described in *Materials and Methods*. PBMC for phenotyping were harvested on the indicated days from parallel 1-ml cultures each inoculated with 10^6 PBMC in Costar #3524 culture plates.

[b] All cpm values are the means of triplicate determinations.

[c] Values in parentheses represent the relative fluorescence intensities for CD25 expressed as the mean channel number for CD25⁺ cells.

[d] Values in parentheses represent the relative fluorescence intensities for CD56 expressed as the mean channel number for CD3⁻56⁺ cells.

tion in the presence of IL-12 is due, at least in part, to enhanced responsiveness to IL-2. On the other hand, culture of PBMC with rIL-2 has been shown to up-regulate expression of IL-12 binding sites on at least a subset of PBMC (B. B. Desai, manuscript in preparation), and, as shown in this report, IL-12 by itself can cause proliferation of IL-2-activated PBMC. It is possible that IL-2-mediated enhancement of IL-12R expression leads to IL-12-induced proliferation of some subset(s) of the PBMC, and that this proliferation is additive to that caused by suboptimal concentrations of IL-2, similar to the results observed when suboptimal amounts of IL-2 and IL-12 were added together to cultures of PHA blasts (Table I). A third mechanism by which IL-12 might enhance the proliferative response of resting PBMC to IL-2 is through the induction of cytokine release in situ. IL-12 can synergize with low concentrations of IL-2 in causing resting PBMC to produce IFN-γ (27) (M. K. Gately, unpublished results) and conceivably other cytokines which might, directly or indirectly, contribute to the observed augmentation of the proliferative response. Moreover, preliminary experiments indicate that the proliferation of PBMC induced by low concentrations of IL-2 can be partially inhibited (35 to 85%) by anti-IL-12 (M. K. Gately, unpublished results), suggesting that IL-2 induces the production of IL-12 in situ. Hence, differences seen in IL-2R expression in cultures receiving IL-2 as compared to IL-2 + IL-12 might have been even greater if the potential effects of endogenously generated IL-12 had been blocked in cultures that received IL-2 alone.

Phenotypic analysis of PBMC cultured in IL-2 or IL-2 + IL-12 from 3 to 9 days revealed a gradual increase in the percentage of CD3⁻56⁺ cells, suggesting that CD56⁺ NK cells constituted the predominant proliferating cell population in these cultures. This observation is consistent with the results of others (10, 12). However, early in cultures containing either IL-2 or IL-2 + IL-12 the percentage of CD25⁺ cells was consistently greater than the percentage of CD3⁻56⁺ cells, raising the possibility that lymphocytes of other subsets may also have proliferated in response to IL-2 or IL-2 + IL-12, at least to a limited extent.

The biologic significance of the multiplicity of cytokines which can stimulate the proliferation of T cells and NK cells is unclear. It may be that the various lymphokine growth factors are differentially produced at different times during the ontogeny of the immune system, at different stages in the development of an immune response, or at different sites within the body or lymphoid tissues. We have previously discussed (24) the possibility that IL-12 may be produced predominantly by B lymphocytes and may constitute a mechanism by which B cells contribute to the amplification of T cell responses (35). Further studies are required to expand our knowledge of the biologic activities of IL-12 and the role which it plays in immune responses.

REFERENCES

1. **Mizel, S. B.** 1989. The interleukins. *FASEB J. 3:2379.*
2. **Cantrell, D. A., and K. A. Smith.** 1984. The interleukin-2 T-cell system: a new cell growth model. *Science 224:1312.*
3. **Mingari, M. C., F. Gerosa, G. Carra, R. S. Accolla, A. Moretta, R. H. Zubler, T. A. Waldmann, and L. Moretta.** 1984. Human interleukin-2 promotes proliferation of activated B cells via surface receptors similar to those of activated T cells. *Nature 312:641.*
4. **Siegel, J. P., M. Sharon, P. L. Smith, and W. J. Leonard.** 1987. The IL-2 receptor β chain (p70): role in mediating signals for LAK, NK, and proliferative activities. *Science 238:75.*
5. **Spits, H., H. Yssel, Y. Takebe, N. Arai, T. Yokota, F. Lee, K-I. Arai, J. Banchereau, and J. E. de Vries.** 1987. Recombinant interleukin 4 promotes the growth of human T cells. *J. Immunol. 139:1142.*
6. **Defrance, T., B. Vanbervliet, J-P. Aubry, Y. Takebe, N. Arai, A. Miyajima, T. Yokota, F. Lee, K-I. Arai, J. E. de Vries, and J. Banchereau.** 1987. B cell growth-promoting activity of recombinant human interleukin 4. *J. Immunol. 139:1135.*
7. **Kawakami, Y., M. C. Custer, S. A. Rosenberg, and M. T. Lotze.** 1989. IL-4 regulates IL-2 induction of lymphokine-activated killer activity from human lymphocytes. *J. Immunol. 142:3452.*
8. **Armitage, R. J., A. E. Namen, H. M. Sassenfeld, and K. H. Grabstein.** 1990. Regulation of human T cell proliferation by IL-7. *J. Immunol. 144:938.*
9. **Londei, M., A. Verhoef, C. Hawrylowicz, J. Groves, P. De Berardinis, and M. Feldmann.** 1990. Interleukin 7 is a growth factor for mature human T cells. *Eur. J. Immunol. 20:425.*
10. **Williams, J. M., M. Abbud-Filho, V. E. Kelley, and T. B. Strom.** 1985. Interleukin-2 apparently up-regulates its receptor and induces proliferation of various resting mononuclear leukocytes in the absence of antigen. *Cell. Immunol. 94:383.*
11. **Bich-Thuy, L. T., M. Dukovich, N. J. Peffer, A. S. Fauci, J. H. Kehrl, and W. C. Greene.** 1987. Direct activation of human resting T cells by IL 2: the role of an IL-2 receptor distinct from the Tac protein. *J. Immunol. 139:1550.*
12. **Caligiuri, M. A., A. Zmuidzinas, T. J. Manley, H. Levine, K. A. Smith, and J. Ritz.** 1990. Functional consequences of interleukin 2 receptor expression on resting human lymphocytes. Identification of a novel natural killer cell subset with high affinity receptors. *J. Exp. Med. 171:1509.*
13. **Welch, P. A., A. E. Namen, R. G. Goodwin, R. Armitage, and M. D. Cooper.** 1989. Human IL-7: a novel T cell growth factor. *J. Immunol. 143:3562.*
14. **Han, X., K. Itoh, C. M. Balch, and N. R. Pellis.** 1988. Recombinant interleukin 4 (rIL 4) inhibits interleukin 2-induced activation of peripheral blood lymphocytes. *Lymphokine Res. 7:227.*
15. **Damle, N. K., and L. V. Doyle.** 1989. Distinct regulatory effects of IL-4 and TNF-α during CD3-dependent and CD3-independent initiation of human T-cell activation. *Lymphokine Res. 8:85.*
16. **Treisman, J., C. M. Higuchi, J. A. Thompson, S. Gillis, C. G. Lindgren, D. E. Kern, S. R. Ridell, P. D. Greenberg, and A. Fefer.** 1990. Enhancement by interleukin 4 of interleukin 2- or antibody-induced proliferation of lymphocytes from interleukin 2-treated cancer patients. *Cancer Res. 50:1160.*
17. **Mizel, S. B.** 1982. Interleukin 1 and T cell activation. *Immunol. Rev. 63:51.*

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18. **Houssiau, F. A., P. G. Coulie, D. Olive, and J. Van Snick.** 1988. Synergistic activation of human T cells by interleukin 1 and interleukin 6. *Eur. J. Immunol. 18:653.*
19. **Uyttenhove, C., R. J. Simpson, and J. Van Snick.** 1988. Functional and structural characterization of P40, a mouse glycoprotein with T-cell growth factor activity. *Proc. Natl. Acad. Sci. USA 85:6934.*
20. **MacNeil, I. A., T. Suda, K. W. Moore, T. R. Mosmann, and A. Zlotnik.** 1990. IL-10, a novel growth cofactor for mature and immature T cells. *J. Immunol. 145:4167.*
21. **Kupper, T., P. Flood, D. Coleman, and M. Horowitz.** 1987. Growth of an interleukin 2/interleukin 4-dependent T cell line induced by granulocyte-macrophage colony-stimulating factor (GM-CSF). *J. Immunol. 138:4288.*
22. **Yokota, S., T. D. Geppert, and P. E. Lipsky.** 1988. Enhancement of antigen- and mitogen-induced human T lymphocyte proliferation by tumor necrosis factor-α. *J. Immunol. 140:531.*
23. **Stern, A. S., F. J. Podlaski, J. D. Hulmes, Y-C. E. Pan, P. M. Quinn, A. G. Wolitzky, P. C. Familletti, D. L. Stremlo, T. Truitt, R. Chizzonite, and M. K. Gately.** 1990. Purification to homogeneity and partial characterization of cytotoxic lymphocyte maturation factor from human B-lymphoblastoid cells. *Proc. Natl. Acad. Sci. USA 87:6808.*
24. **Gubler, U., A. O. Chua, D. S. Schoenhaut, C. M. Dwyer, W. McComas, R. Motyka, N. Nabavi, A. G. Wolitzky, P. M. Quinn, P. C. Familletti, and M. K. Gately.** 1991. Coexpression of two distinct genes is required to generate secreted bioactive cytotoxic lymphocyte maturation factor. *Proc. Natl. Acad. Sci. USA 88:4143.*
25. **Gately, M. K., D. E. Wilson, and H. L. Wong.** 1986. Synergy between recombinant interleukin 2 (rIL 2) and IL 2-depleted lymphokine-containing supernatants in facilitating allogeneic human cytolytic T lymphocyte responses in vitro. *J. Immunol. 136:1274.*
26. **Wong, H. L., D. E. Wilson, J. C. Jenson, P. C. Familletti, D. L. Stremlo, and M. K. Gately.** 1988. Characterization of a factor(s) which synergizes with recombinant interleukin 2 in promoting allogeneic human cytolytic T-lymphocyte responses in vitro. *Cell. Immunol. 111:39.*
27. **Kobayashi, M., L. Fitz, M. Ryan, R. M. Hewick, S. C. Clark, S. Chan, R. Loudon, F. Sherman, B. Perussia, and G. Trinchieri.** 1989. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. *J. Exp. Med. 170:827.*
28. **Chan, S. H., B. Perussia, J. W. Gupta, M. Kobayashi, M. Pospisil, H. A. Young, S. F. Wolf, D. Young, S. C. Clark, and G. Trinchieri.** 1991. Induction of interferon γ production by natural killer cell stimulatory factor: characterization of the responder cells and synergy with other inducers. *J. Exp. Med. 173:869.*
29. **Dower, S. K., S. H. Hefeneider, A. R. Alpert, and D. L. Urdal.** 1985. Quantitative measurement of human interleukin 2 receptor levels with intact and detergent-solubilized human T-cells. *Mol. Immunol. 22:937.*
30. **Tsudo, M., F. Kitamura, and M. Miyasaka.** 1989. Characterization of the interleukin 2 receptor β chain using three distict monoclonal antibodies. *Proc. Natl. Acad. Sci. USA 86:1982.*
31. **Gately, M. K., G. Glaser, S. J. Dick, R. W. Mettetal, Jr., and P. L. Kornblith.** 1982. In vitro studies on the cell-mediated immune response to human brain tumors. I. Requirement for third-party stimulator lymphocytes in the induction of cell-mediated cytotoxic responses to allogeneic cultured gliomas. *JNCI 69:1245.*
32. **Sinigaglia, F., M. Guttinger, P. Romagnoli, and B. Takacs.** 1990. Malaria antigens and MHC restiriction. *Immunol. Letters 25:265.*
33. **Kilgus, J., T. Jardetsky, J. C. Gorga, A. Trzeciak, D. Gillessen, and F. Sinigaglia.** 1991. Analysis of the permissive association of a malaria T cell epitope with DR molecules. *J. Immunol. 146:307.*
34. **Wysocki, L. J., and V. L. Sato.** 1978. "Panning" for lymphocytes: a method for cell selection. *Proc. Natl. Acad. Sci. USA 75:2844.*
35. **Liu, Y., and A. Mullbacher.** 1989. Hypothesis: immunological help is reciprocally delivered between different subpopulations of lymphocytes. *Scand. J. Immunol. 30:277.*

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