Selective Generation of Erythroid Burst–Promoting Activity by Recombinant Interleukin 2–Stimulated Human T Lymphocytes and Natural Killer Cells

By Sandra Skettino, Joseph Phillips, Lewis Lanier, Arnon Nagler, and Peter Greenberg

Because T lymphocytes and natural killer (NK) cells produce a variety of growth factors and interleukin 2 (IL2) modulates the activity of both, we assessed the ability of IL2 to stimulate human T cells and NK cells to produce hematopoietic growth factors detectable in clonogenic marrow culture. Human recombinant interleukin 2 (rIL2) added directly to cultures of human bone marrow that had been depleted of monocytes or depleted of both monocytes and T cells caused no significant alteration of myeloid (CFU-GM) or erythroid colony formation. Conditioned media harvested from rIL2-stimulated (>100 U/mL) peripheral blood mononuclear cells, T cells, Leu-2 cells, and Leu-3 cells all had erythroid burst–promoting activity (BPA) but lacked myeloid colony–stimulating factor (GM-CSF) or CFU-GM–inhibitory activity. These T cells were IL2 receptor–negative, and the addition of anti-IL2 receptor monoclonal antibody (anti-Tac) to T cell cultures did not abrogate this IL2-stimulated BPA production. In addition, Percoll gradient–enriched, large granular lymphocytes (LGL) were separated by fluorescence-activated cell sorting into Leu-11+ (NK) cells and Leu-11− (low-density Leu-4+ T) cell fractions. rIL2 stimulated LGL, Leu-11+ and Leu-11− cells to produce BPA but not detectable GM-CSF or CFU-GM–inhibitory activity. Leu-11+ (NK) cells were Tac-negative from days 0 through 14 of culture. We conclude that rIL2 at high concentrations stimulated T cells, Leu-2 and Leu-3 cell subsets, LGL, and NK cells to produce BPA but not GM-CSF and that this stimulation may be mediated by an IL2 receptor distinct from Tac or by an epitope of the IL2 receptor not recognized by the anti-Tac antibody.

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PRIOR STUDIES HAVE SHOWN that cells involved in the immune response, specifically T cells and natural killer (NK) cells, produce substances capable of regulating hematopoiesis.[1-7] The activity of T cells is modulated in part by interleukin 2 (IL2), a 15-kd glycoprotein produced by helper T cells in response to antigenic or mitogenic activation.[8] Activation of resting T cells results not only in IL2 secretion but also in the expression of a specific cell surface receptor for IL2 that is recognized by the monoclonal antibody anti-Tac.[9,10] IL2 interacts with this receptor to induce T cell proliferation as well as lymphokine release.[11-13] Although the immune functions of IL2 appear to be mediated by the high-affinity receptor,[14,15] the function of the low-affinity receptor remains unknown. Recently it has been reported that T cells may respond to IL2 in the absence of demonstrable Tac receptor,[13,16] which suggests an alternative mechanism of T cell regulation by IL2.

Similarly, several studies have suggested that large granular lymphocytes (LGL), including NK cells, may play a role in the regulation of hematopoiesis. Controversy exists in the literature regarding the role of LGL and NK cells for hematopoiesis because these cells have been reported to exert either stimulatory[6] or inhibitory effects in vitro.[4,5,7] These disparate results appear to be due in part to the heterogeneity of the LGL population evaluated. Anti–Leu-11 monoclonal antibody recognizes the Fc receptor present on NK cells and neutrophils and may be used to separate LGL into Leu-11+/4− NK cells and Leu-11+/4− low-density T cells.[17,18] The Leu-11–positive cells contain virtually all the cells with NK activity.[17] IL2 has been reported to exert immunoregulatory effects on NK cells including augmenting NK cytotoxic activity,[19-22] inducing interferon production from NK cells,[19,22] and stimulating NK cell proliferation.[19,22] Anti-Tac monoclonal antibody is unable to block these effects of IL2,[19,20,22] again suggesting a Tac-independent mechanism of IL2 regulation. We have used purified populations of T cells, NK cells, bone marrow (BM) target cells and human recombinant IL2 (rIL2) to further clarify the roles of these cells, IL2, and the IL2 receptor in regulating hematopoiesis.

METHODS

Marrow and blood samples. BM samples were obtained from normal controls according to guidelines established by the Stanford University Human Experimentation Committee. Three to 5 mL marrow aspirate was drawn into a heparinized syringe. Buffy coats prepared from the peripheral blood cells (PB) of normal volunteer blood donors were obtained from the Stanford University Blood Bank. BM or buffy coat samples were diluted with phosphate-buffered saline (PBS), layered over Ficoll-Hypaque (density, 1.077 g/mL), and centrifuged at 400 g for 20 minutes, and the interface buoyant mononuclear cells (MNC) were collected, washed, and counted.

Monoclonal antibodies. Anti–Leu-1 and anti–Leu-4 pan-T monoclonal antibodies, anti–Leu-2a (which recognizes the suppressor/cytotoxic T subset), anti–Leu-3a (which recognizes the helper/inducer T subset), anti–Leu-M3 (which recognizes monocytes) and anti–My-10 (which recognizes hematopoietic progenitor cells) were purchased from Becton Dickinson, Inc, Mountain View, CA. Anti-Leu-11, which recognizes the Fc receptor on neutrophils and NK cells,[17] was provided by Dr Lewis Lanier, Becton Dickinson. Goat antimouse IgG, unconjugated and fluorescein conjugated, were purchased from Tago, Inc, Burlingame, CA. Anti-Tac (anti-IL2 receptor) ascites containing 6 to 8 mg/mL anti-Tac antibody[9,10] was the kind gift of Dr Thomas Waldmann, National Institutes of

Health, Bethesda, MD. To assess the potency of the anti-Tac antibody, this material was added to T cells (0.5 × 10⁶ cells/mL in RPMI + 10% fetal calf serum [FCS]) for 30 minutes at 23°C before culture. IL2, 100 U/mL, was then added, and triplicate cultures were incubated for 72 hours in humidified 5% CO_2. T cell proliferation was measured by ³H-thymidine incorporation as previously described.[10] Anti-Tac ascites fluid at a 1:100 dilution inhibited 83% of the ³H-thymidine uptake seen in control T cell cultures containing IL2 alone (control = 11,744 ± 398 cpm; mean ± SE; m = 3); anti-Tac at a 1:1,000 dilution inhibited 63% of the control response.

IL2. Human recombinant IL2 from *Escherichia coli* (5.2 × 10⁶ U/mg, lot LP241)[23,24] was kindly provided by Dr Kirsten Koths, Cetus Corp, Emeryville CA.

Indirect immunofluorescence. A total of 1 × 10⁶ cells were incubated with 75 μL of the appropriate mouse antihuman monoclonal antibody for 20 minutes at 4°C and then washed in PBS plus 0.02% azide. The cells were then incubated with 75 μL fluoresceinated goat antimouse IgG for 20 minutes at 4°C and washed in PBS–0.02% azide as previously described.[25] The percentage of fluorescent cells (100 to 300 cells per sample) was counted by using a Leitz Ortholux II fluorescent microscope.

Double adherence. BM-MNC were suspended at a concentration of 10⁷ cells/mL in Iscove's modified Dulbecco's medium (IMDM) with 7.5% FCS (Irvine Scientific, Santa Ana, CA) and incubated on Petri dishes at 37°C for 45 minutes. The nonadherent cells were removed and the aforementioned procedure repeated. After the second incubation, the nonadherent buoyant cells (NAB) were removed by gentle rinsing. BM-MNC after double adherence were <1% Leu-M3–positive by indirect immunofluorescence.

Erythrocyte rosetting. Sheep RBC (SRBC) were incubated with neuraminadase for one hour, washed, diluted to a 5% suspension, and mixed with an equal volume of 10⁷/mL PB-MNC. This mixture was underlayed with Ficoll-Hypaque, incubated for 60 minutes at 4°C, and centrifuged at 400 g for 25 minutes. The SRBC in the rosetted pellet were lysed by using a 0.87% NH_4Cl solution and the remaining cells washed in IMDM plus 15% FCS.

Antibody-mediated plate binding ("panning"). As previously described,[25] Fischer 100 × 15-mm polystyrene plates were coated with 100 μg purified goat antimouse IgG in 10 mL 0.05 mol/L Tris buffer, pH 9.5. After incubating for 40 minutes at room temperature, the buffer was decanted, the plates were washed five times with PBS, 5 mL of PBS–1% FCS was added, and the plates were stored at 4°C for a maximum of 24 hours. NAB-BM cells or T cells rosetted from PB were incubated with 75 μg of the appropriate monoclonal antibody for 20 minutes at 4°C. These coated cells were then suspended in 3 mL PBS–5% FCS and poured onto the antibody-coated plate. The plates were incubated on a level surface at 4°C for 70 minutes, with the plate swirled once at 40 minutes to redistribute the cells. Nonbound cells were recovered by gentle washing with PBS–1% FCS. Bound cells were recovered by vigorously flushing the plate with a 25-gauge needle attached to a 30-mL syringe. NAB-BM cells, after panning with Leu-1 to remove the T cells, were <1% Leu-1–positive by indirect immunofluorescence.

Enrichment procedure for LGL, NK, and hematopoietic precursor cells. LGL and NK cells were obtained essentially as previously described.[17,26] PB-MNC were depleted of monocytes by adherence to plastic. B cells and residual adherent cells were removed by twice passing the cells through a nylon wool column. These nylon wool–nonadherent cells were placed in a 30% Percoll solution and then layered over 40% Percoll, and after centrifugation for 50 minutes the low-density interface containing LGL was harvested. The LGL were labeled with fluorochrome-conjugated, Leu-11 monoclonal antibody and separated by fluorescence-activated cell sorting (FACS 440, Becton Dickinson FACS Division, Sunnyvale, CA) as previously described[17] into Leu-11+ (NK cells) and Leu-11–

low-density T cells.[17] The Percoll gradient–separated LGL were approximately 50% Leu-11+/4– and 50% Leu-4+/Leu-11–. The FACS-separated NK cells were >98% Leu-11+ by phenotype reanalysis using flow cytometry. The Leu-11– population was >95% Leu-4+ and Leu-1+ by phenotype reanalysis by FACS. Microscopic assessment of immunofluorescence was comparable to FACS because Leu-11– cells labeled with anti–Leu-1 antibody were found to be 95% Leu-1+ (100 of 110 cells scored).

Hematopoietic precursor cells including CFU-GM, BFU-E, and CFU-GEMM have a cell surface antigen defined by the monoclonal antibody My10.[27] PB-MNC depleted of adherent cells were layered onto a Percoll gradient as described earlier. The light-density interface cells were harvested, labeled with fluorochrome-conjugated My10 monoclonal antibody, and separated by FACS into My10+ and My10– populations. Of the cells sorted 4% ± 2% were My10+. Reanalysis of this cell fraction showed that these cells were >95% My10+ by FACS.

Conditioned media preparation. MNC from PB or BM, after undergoing the appropriate separation procedures, were suspended at a concentration of 10⁶ cells/mL in IMDM containing 15% FCS, 0.5 mmol/L 2-mercaptoethanol (2ME), and 1% penicillin/streptomycin. IL2 was added to some cultures at concentrations of 10 to 1,500 U/mL. The cultures were incubated at 37°C in 5% CO_2 and the cells and supernatants harvested at days 2 through 14.

Assay for CFU-GM and BFU-E. BM-MNC (2.0 × 10⁵ cells/mL) that were depleted of monocytes (NAB) or depleted of monocytes and T cells (NAB-T) were cultured in IMDM with 15% FCS, 0.9% bovine serum albumin (Armour Pharmaceutical Co, Tarrytown, NY), 50 μmol/L 2ME, 1% penicillin/streptomycin, 1% L-glutamine, 0.5 U/mL purified human urinary erythropoietin (1,140 U/mg protein obtained from Alan Eaves, British Columbia Cancer Center), and methylcellulose (final concentration, 1.1%) as previously described.[25] The cultures were seeded in duplicate in 0.25-mL volumes in Costar Mark II tissue culture plates (Costar, Cambridge, MA). Control plates for BFU-E contained this culture medium alone. Conditioned media (CM) that were tested were added at a final concentration of 15%. Some cultures of My10+ cells also contained 1% MoCM (a T cell line CM kindly provided by Dr David Golde, UCLA Medical Center) as a burst-promoting activity (BPA) source. After 14 days of incubation in humidified 5% CO_2 at 37°C the cultures were examined under an inverted microscope and BFU-E colonies scored.

Cultures for CFU-GM were plated as before, but omitting erythropoietin from the culture medium. CM were added at a final concentration of 15%. Placental CM (PCM), prepared as previously described,[25] was added to control cultures at a final concentration of 15% as an exogenous colony-stimulating factor (CSF) source. CFU-GM colonies were scored on day 10.

Statistics. Results were analyzed for statistical significance by using Student's *t* test and, in the case of paired samples, the paired Student's *t* test.

RESULTS

Effect of rIL2 on the growth of enriched BM cells and PB hematopoietic precursors. We assessed the ability of rIL2 to stimulate BFU-E and CFU-GM colony formation by the target BM cells. Because IL2 is a mediator of T-cell function, we determined whether the presence of T cells in the target BM affected colony formation on exposure to IL2. rIL2 in concentrations of 1.5 to 1,500 U/mL was added to cultures of BM-MNC that had been depleted of monocytes (NAB) or depleted of monocytes and T cells (NAB-T). As shown in Fig 1, BFU-E growth in the presence of IL2 was not significantly
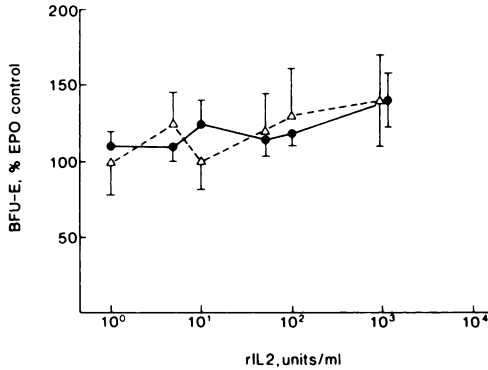
Fig 1. Marrow BFU-E response to IL2. BFU-E are expressed as a percentage of BFU-E in cultures that contained 0.5 U/mL erythropoietin alone. IL2 concentrations shown are the final concentrations in culture. Results shown are means ± SE. Numbers of BFU-E per 1 × 10⁵ cells in control cultures containing erythropoietin alone were 49 ± 9 (mean ± SE; n − 4) for NAB BM and 57 ± 9 (mean ± SE; n − 6) for NAB-T BM. — NAB (n − 4); --- NAB-T (n − 4).
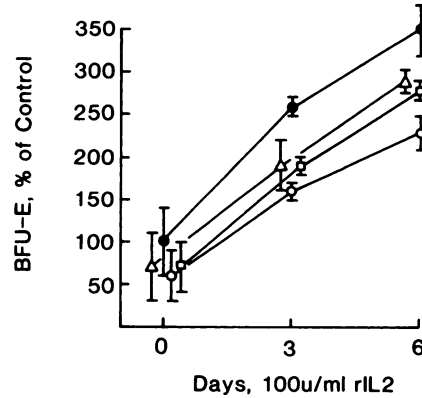

Fig 2. BPA production by IL2-stimulated T cells. CM from cell subsets stimulated with rIL2, 100 U/mL, were harvested at days 3 and 6 and cultured at a 15% concentration with NAB-T BM. BFU-E are expressed as a percentage of BFU-E in cultures that contained 0.5 U/mL erythropoietin alone. Values represent means ± SE of duplicate or triplicate experiments. Numbers of BFU-E per 1 × 10⁵ cells in control cultures containing erythropoietin alone were 27 ± 4 (mean ± SE). ● MNC; ○ T cells; □ Leu 2 cells; △ Leu 3 cells.

different from control cultures grown in the presence of erythropoietin alone when using either NAB or NAB-T target BM cells. No CFU-GM colony growth was detected from either NAB or NAB-T BM stimulated by IL2, nor was CFU-GM inhibition seen when PCM (as a CSF source) was added to the IL2-containing cultures (data not shown).

To more directly determine whether rIL2 would alter colony formation by hematopoietic precursor cells, PB nonadherent light-density My10+ cells were obtained as described earlier. rIL2, 100 U/mL, added to cultures of My10+ cells did not stimulate BFU-E or CFU-GM growth. Further, as shown in Table 1, the addition of 100 U/mL rIL2 to cultures of My10+ cells containing either PCM or MoCM plus erythropoietin did not alter either CFU-GM or BFU-E growth.

Stimulation by rIL2 of hematopoietic growth factors from T cells and T cell subsets. To more directly assess the ability of IL2 to stimulate T cell production of hematopoietic growth factors, T cells were obtained from PB-MNC by

rosetting and further subdivided into Leu-2 and Leu-3 subsets by indirect panning as described earlier. These cell fractions were cultured with rIL2 (100 U/mL), and CM was harvested on days 3 and 6. These CM were cultured at a 15% concentration with NAB-T BM target cells. As shown in Fig 2, rIL2 stimulated MNC, T cells, and Leu-2 and Leu-3 subsets to produce BPA on day 3, with slightly increased BPA production on day 6.

These cell subsets were then exposed to various doses of IL2 (10 to 1,000 U/mL), three-day CM were harvested, and these CM were cultured at a 15% concentration with NAB-T target BM cells. As shown in Fig 3, low concentrations of IL2 (10 U/mL) stimulated BPA production from MNC but not from T cells or Leu-2 or Leu-3 subsets, whereas concentra-

Table 1. Effects of Human rIL2 on My10+ PB Hematopoietic Precursor Cell Growth

Experiment	rIL2 (100 U/mL)	CFU-GM*	BFU-E†
1	−	178 ± 5	251 ± 20
	+	170 ± 8	251 ± 14
2	−	149 ± 54	837 ± 80
	+	162 ± 27	783 ± 100

PB nonadherent light-density My10+ cells, obtained as described, were used as target cells for hematopoietic colony formation by plating 3.75 × 10⁴ or 3.75 × 10³ cells in duplicate in 0.25 mL methylcellulose medium within Costar microwells. Values are means ± SE.

*CFU-GM colonies/1 × 10⁵ cells plated were stimulated with 15% PCM and counted on day 10. No colonies formed with or without rIL2 (100 U/mL) in the absence of PCM.

†BFU-E colonies/1 × 10⁵ cells plated were stimulated with 1% MoCM plus 0.5 U/mL erythropoietin and counted on day 14. No colonies formed with or without rIL2 (100 U/mL) in the absence of MoCM plus erythropoietin.
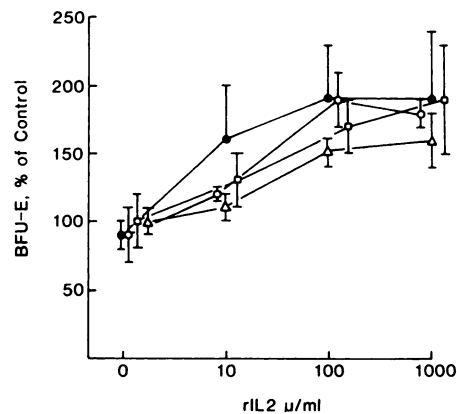

Fig 3. BPA production by T cells stimulated with increasing doses of IL2. CM from cell subsets stimulated with increasing doses of IL2 were harvested at day 3 and cultured at a 15% concentration with NAB-T BM. BFU-E are expressed as a percentage of BFU-E in cultures containing 0.5 U/mL erythropoietin alone. Values represent means ± SE of duplicate or triplicate experiments. Numbers of BFU-E per 1 × 10⁵ cells in control cultures containing erythropoietin alone were 22 ± 2 (mean ± SE). ● MNC; ○ T cells; □ Leu 2 cells; △ Leu 3 cells.

tions of 100 U/mL or greater stimulated significant BPA production from all cell fractions.

Figure 4 shows growth factor production from PB-MNC, T cells, and Leu-2 and Leu-3 subsets. These cell subsets were stimulated with IL2, 100 U/mL, three-day CM were harvested, and these CM were cultured with BM NAB-T target cells. IL2 generated statistically significant increases in BPA production from all subsets tested (MNC, n = 8, $P < .01$; T cells, n = 13, $P < .001$; Leu-2 cells, n = 8, $P < .02$; Leu-3 cells, n = 9, $P < .05$). There was no difference in the level of IL2-stimulated BPA production between T cells or Leu-2 or Leu-3 cells. No CFU-GM formed when CM alone was added to these cultures, thus indicating that no GM-CSF was produced by these stimulated subsets (Fig 4). No inhibition of CFU-GM growth was observed when these CM were cultured with PCM as an exogenous CSF source (data not shown).

IL2 stimulation of growth factors from PB v BM T cells. To compare PB T cells to the possibly more physiologically relevant BM T cells, T cells were obtained from PB by rosetting and from BM by panning with Leu-1 monoclonal antibody. T cells were cultured with increasing doses of IL2 (10 to 1,000 U/mL), three-day CM were harvested, and these CM were cultured with NAB-T target BM cells. As shown in Fig 5, there was no significant difference between PB and BM T cell BPA production in response to IL2. Again, little BPA production was demonstrable at low IL2 concentrations, whereas significant BPA production was seen at IL2 concentrations of 100 U/mL or greater.

Fig 5. Erythroid BPA generation by IL2-stimulated BM and PB T cells. BFU-E are expressed as a percentage of BFU-E in cultures containing 0.5 U/mL erythropoietin alone. CM from PB and BM T cells stimulated with increasing doses of IL2 were harvested at day 3, and CM were cultured with NAB-T target BM cells. Results shown are means ± SE. Numbers of BFU-E per 1 x 10⁵ cells in control cultures containing erythropoietin alone were 60 ± 10 (mean ± SE, n = 4). — BM T-cells (n = 3); --- PB T-cells (n = 4).

Effect of anti-Tac antibody on IL2-stimulated BPA production. BM and PB T cells in the aforementioned experiments were less than 1% positive for IL2 receptors by indirect immunofluorescence using anti-Tac monoclonal antibody on days 0, 3, and 6 of culture in the presence or absence of IL2. To more directly assess the role of the Tac receptor in IL2-related stimulation of BPA production, T cells were preincubated for 60 minutes with anti-Tac ascites in dilutions ranging from 1:100 to 1:10⁶. IL2, 100 U/mL, was then added to these cultures containing anti-Tac, and three-day CM were harvested. CM from control cultures that contained anti-Tac but no added IL2 were also obtained. These CM were cultured with NAB-T BM. As shown in Table 2, anti-Tac antibody did not block BPA production from either unstimulated T cells or T cells stimulated with IL2.

Hematopoietic growth factor production by IL2-stimulated LGL and NK cells. To assess the ability of IL2 to stimulate LGL and NK cells to produce BPA, Percoll-separated LGL were further subdivided by FACS separation into Leu-11+/4− NK cells and Leu-11−/4+ low-density T cells. These cells were incubated with increasing doses of IL2, three-day CM were harvested, and these CM were cultured with NAB-T BM cells. rIL2, 10 U/mL, stimulated BPA production from Leu-11+ cells, whereas rIL2, 100 U/mL or greater, stimulated significant BPA production from all cell fractions (Fig 6).

LGL, NK cells, and low-density T cells were cultured in IL2, 1,500 U/mL, CM were harvested on days 2 through 14, and these CM were cultured with NAB-T BM. BPA production increased with time in all cell fractions as shown in Fig 7. Again, no GM-CSF nor CFU-GM–inhibitory activity was detected in these CM. Leu-11+ (NK cells) did not express IL2 receptor as determined by FACS analysis using anti-Tac monoclonal antibody on days 1 through 14 of culture.
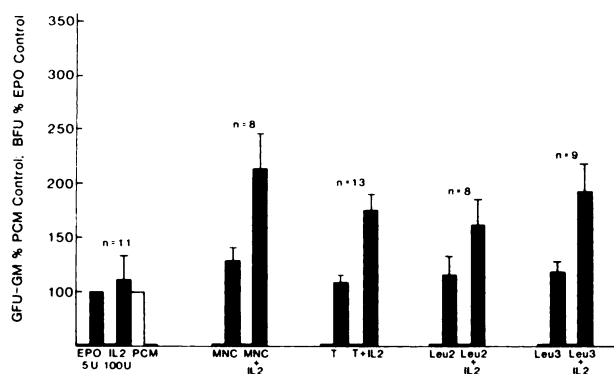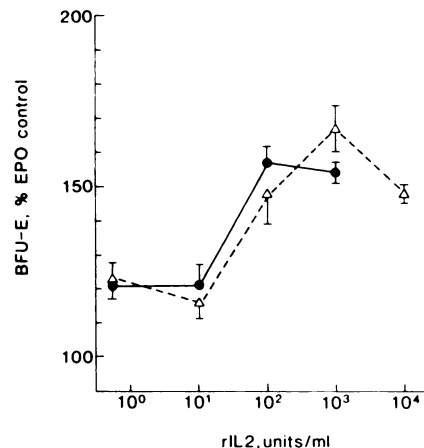

Fig 4. Hematopoietic growth factor production by IL2-stimulated PB subsets. CM from cell subsets stimulated with rIL2, 100 U/mL, were harvested at day 3 and cultured at a 15% concentration with NAB-T BM. Cultures that were assayed for BPA production contained CM and 0.5 U/mL erythropoietin. BFU-E are expressed as a percentage of BFU-E in cultures containing 0.5 U/mL erythropoietin alone. Cultures that were assayed for CSF production contained CM alone. Fifteen percent PCM was added to CFU-GM control cultures only. No CFU-GM were detected in cultures containing CM alone. Open bars represent CFU-GM; solid bars represent BFU-E. Results shown are means ± SE. Numbers of BFU-E per 1 x 10⁵ cells in control cultures containing erythropoietin alone were (mean ± SE) 29 ± 4 for MNC and Leu-2 cells (n = 8), 50 ± 10 for T cells (n = 13), and 28 ± 9 for Leu-3 cells (n = 9). Numbers of CFU-GM per 1 x 10⁵ cells in control cultures containing 15% PCM were (mean ± SE) 87 ± 8 for MNC and Leu-2 cells (n = 8), 104 ± 9 for T cells (n = 13), and 93 ± 9 for Leu-3 cells (n = 9). ■ BFU-E; □ GM.

Table 2. Effect of Anti-Tac Antibody on BPA Production by IL2-Stimulated T Cells

Cells Generating CM*	Anti-Tac Antibody Ascites Dilution†	BFU-E, Percentage of Erythropoietin Control‡
T cells	0	121 ± 4
	1:10²	112 ± 3
	1:10³	119 ± 8
	1:10⁴	122 ± 5
	1:10⁵	114 ± 11
	1:10⁶	122 ± 5
T cells + IL2	0	159 ± 3
(100 U/mL)	1:10²	157 ± 9
	1:10³	156 ± 6
	1:10⁴	162 ± 8
	1:10⁵	153 ± 7
	1:10⁶	157 ± 4

*Day 3 CM tested against NAB-T BM.

†Dilutions represent the final dilution of anti-Tac ascites in culture.

‡BFU-E are expressed as a percentage of BFU-E in cultures containing erythropoietin, 0.5 U/mL, alone. Values are means ± SE (n = 3). Numbers of BFU-E per 1 × 10⁵ cells in control cultures containing erythropoietin alone were 92 ± 17 (mean ± SE).

Leu-11 − (low-density T cells) were initially negative for IL2 receptor, but 50% of the cells became Tac antigen–positive on day 7 of culture.

Effect of NK cell depletion on IL2-stimulated BPA production by T lymphocytes. Because NK cells are present in small numbers in the T cell effector fractions as well as in the target BM population, we investigated the possibility that the IL2-stimulated BPA in the T cell CM was being produced by NK cells. NK cells were depleted from SRBC-

Fig 7. Erythroid BPA production by IL2-stimulated LGL and NK cells. BFU-E are expressed as a percentage of BFU-E in cultures containing 0.5 U/mL erythropoietin alone. CM from LGL and Leu-11+ and Leu-11− cells stimulated with IL2, 1,500 U/mL, were harvested at the days indicated and cultured with NAB-T BM. Values shown are means ± SE of duplicate experiments. Numbers of BFU-E per 1 × 10⁵ cells in control cultures containing erythropoietin alone were 35 ± 14 (mean ± SE).

rosetted PB T cells or NAB-T BM cells by panning with Leu-11 monoclonal antibody. Residual NK cell contamination was <1% as detected by indirect immunofluorescence with Leu-11. PB T cells both before and after Leu-11 panning were incubated with IL2, 100 U/mL, and three-day CM were harvested. These CM were cultured with NAB-T BM cells and NAB-T BM cells that had also been depleted of NK cells (NAB-T–Leu-11). As shown in Fig 8, depletion of NK cells from either the T cell effector or the NAB-T target BM populations did not affect BPA production, thus indicat-
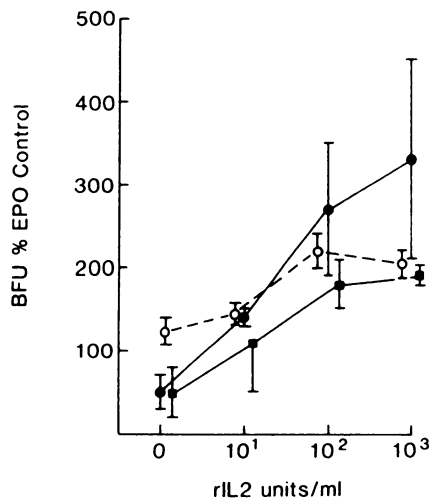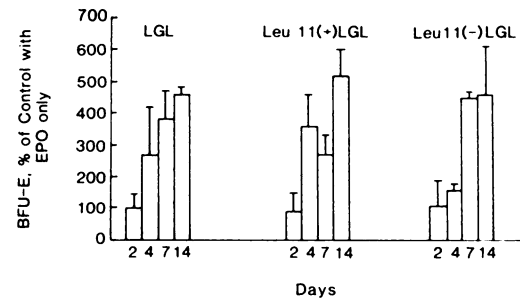

Fig 6. Erythroid BPA generated by IL2-stimulated LGL, NK, and low-density T cells. BFU-E are expressed as a percentage of BFU-E in cultures containing 0.5 U/mL erythropoietin alone. CM from LGL, NK cells (Leu 11+/4−), and low-density T cells (Leu 11−/4+) stimulated with increasing doses of IL2 were harvested at day 3 and cultured with NAB-T target BM cells. Values represent means ± SE of duplicate experiments. Numbers of BFU-E per 1 × 10⁵ cells in control cultures containing erythropoietin alone were 58 ± 10 (mean ± SE). O—O LGL; ●—● Leu 11+/Leu 4— (NK); ■—■ Leu 11 − /Leu 4+ (low density T).
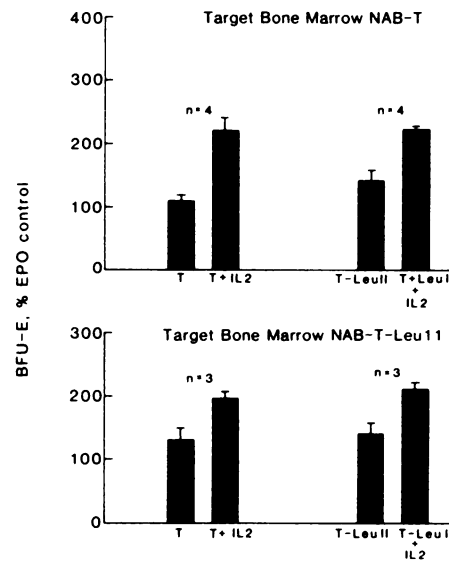

Fig 8. Erythroid BPA production by IL2-stimulated T cells before and after Leu-11 cell depletion. BFU-E are expressed as a percentage of BFU-E in cultures containing 0.5 U/mL erythropoietin alone. (Top) Three-day CM from T cells before and after Leu-11 depletion were stimulated with IL2, 100 U/mL, and cultured with NAB-T BM. (Bottom) The same CM were cultured with NAB-T Leu-11–depleted BM. Results shown are means ± SE. Numbers of BFU-E per 1 × 10⁵ cells in control cultures containing erythropoietin alone were (mean ± SE) 22 ± 3 for NAB-T BM (n = 3) and 39 ± 9 for NAB-T–Leu-11 BM (n = 3).

ing that IL2-stimulated T lymphocytes could provide BPA independent of NK cells.

DISCUSSION

Studies in the past have shown that T cells and NK cells are capable of regulating hematopoiesis and that their functions are modulated by IL2. In our studies we have demonstrated a role for IL2 in regulating human hematopoiesis in vitro. Our data showed that human rIL2 did not stimulate hematopoietic colony formation when added directly to cultures of BM or PB nonadherent, light-density, My10+-enriched precursor cells, which suggests that rIL2 did not directly stimulate BFU-E proliferation. In contrast, IL2 at relatively high concentrations (ie, >100 U/mL), stimulated BPA production by T cells, Leu-2 and Leu-3 subsets, LGL, and NK cells. These subsets, which define immunologic function, had similar potency in producing BPA. This indicated an indirect role for IL2 on hematopoiesis by its ability to stimulate accessory cell production of BPA. In vivo high concentrations of IL2 may be generated locally within bone marrow to stimulate these cells to produce BPA. There was no significant difference in the ability of T cells from BM v those from PB to produce BPA in response to IL2.

Interestingly, no GM-CSF or CFU-GM–inhibitory activity was detected in CM from these stimulated cell fractions. It is possible that low levels of CSF may have been produced that were below the limit of detection of our bioassay because CM prepared with high (5 × 10^6/mL) T cell concentrations stimulated low numbers of CFU-GM colonies (data not shown). Gasson et al recently reported that resting T cells did not express the gene for human GM-CSF as determined by RNA analysis and radioimmunoassay.[28] The expression of this gene in T cells stimulated with IL2 would provide definitive evidence for the ability of IL2 to stimulate CSF production by T lymphocytes.

In our studies the addition of IL2 in concentrations ranging from 1.5 to 1,500 U/mL to BM cells that had been depleted of either adherent cells (mainly monocytes) or of both adherent cells and T cells did not stimulate CFU-GM colony formation or increase BFU-E colony formation over control cultures stimulated by erythropoietin alone. CM obtained from IL2-stimulated T cells, T cell subsets, LGL, and NK cells, however, all contained significant amounts of BPA. This apparent disparity may relate to the fact that T cell and NK cell populations are present in low numbers in BM. Small numbers of T cells, LGL, and NK cells may produce quantities of BPA below the limits of detectability in this assay. The CM, conversely, were generated from relatively homogeneous cell populations at higher cell concentration (10^6/mL). The lack of BFU-E growth in IL2-containing BM cultures may also relate to the presence of endogenous accessory cell populations within the unseparated marrow that may have competing influences on marrow growth in the presence of IL2.

A recent report[29] differs from ours in its finding that concentrations of 1.0 to 10 U/mL IL2 stimulated CFU-E and BFU-E growth in vitro. It is possible that the erythroid colony growth reported by these authors may be a result of the presence of increased numbers of accessory cells in their target marrow population. Small numbers of residual monocytes remaining even after adherence separation procedures produce IL1, which may then stimulate T cells to produce growth factors. Also differing from our findings, the same study[29] reported that the addition of anti-Tac monoclonal antibody to BM cultures (without exogenous IL2) caused a dose-dependent decrease in erythroid colony formation. This finding appears surprising in view of the absence of Tac receptors on most resting T cells. Inhibition of erythroid growth by the anti-IL2 receptor antibody suggests that their culture system had been stimulated to produce endogenous IL2 or that their T cells had been activated.

Previous studies have indicated that LGL and NK cells modulate hematopoiesis, but conflicting reports have demonstrated either stimulatory or inhibitory effects of these cells on BM cell proliferation.[4-7] The dispute in the literature, in part, may relate to the heterogeneity of cell types contained within the LGL, NK, or null cell populations used in prior studies. Our data demonstrate that highly purified, FACS-separated Leu-11+ NK cells can be stimulated with IL2 to produce BPA. Degliantoni et al[7] used monoclonal antibody B73.1 for cell separation which, like Leu-11, recognizes the Fc receptor on NK cells. Their results demonstrated inhibition of early CFU-GM and CFU-E only when BM cells were preincubated with B73.1+ cells or when BM cells were cultured with supernatants from B73.1+ cells that had been stimulated with γ-interferon. Although studies showed that NK cells stimulated with IL2 produce BPA, cell-cell contact or γ-interferon stimulation may result in the production of NK cytotoxic factor,[30] interferon, or other substances inhibitory for hematopoiesis. This implies a complex mechanism of hematopoietic regulation by NK cells whereby different modes of NK cell activation would result in the production of factors that would either stimulate or inhibit hematopoiesis.

In these experiments we also investigated the role of the IL2 receptor by determining whether blocking the IL2 receptor with anti-Tac monoclonal antibody would influence IL2-stimulated BPA production by T cells and NK cells. Our data show that purified T cells and NK cells were Tac antigen–negative both before and after IL2 stimulation as determined by immunofluorescence. Although unstimulated T cells may express low levels of IL2 receptor[15] that are below the limit of detectability of immunofluorescence, incubation with various concentrations of anti-Tac monoclonal antibody did not interfere with BPA production by either unstimulated T cells or T cells that had been stimulated with IL2. Conversely, anti-Tac in the same concentrations inhibited T cell proliferation as assessed by ^3H-thymidine uptake. These data suggest that IL2-stimulated BPA production is mediated by a non-Tac IL2 receptor or by an epitope of the IL2 receptor not recognized by anti-Tac. Other investigators have reported effects of IL2 that appear to occur independently of the Tac epitope of the IL2 receptor such as proliferation[16] and IL3 production[13] by Tac-negative T lymphocytes in response to the IL2. Anti-Tac antibody also did not diminish IL2-mediated enhancement of NK cell cytotoxicity.[19] In this regard, a non-Tac peptide present on human T cells and LGL that binds IL2 has recently been identified and characterized.[31-33] This 70- to 75-kd protein,

which lacks reactivity with the anti-Tac antibody, binds IL2 with intermediate affinity (K_d approximately equal to 1 nmol/L) and has been proposed to participate with the Tac antigen in the formation of high-affinity IL2 receptors.[33] The low- and high-affinity IL2 receptors on PB lymphocytes have affinity constants of 36 nmol/L and 8.5 pmol/L, respectively.[15] In view of these data, our findings that BPA production was seen at IL2 concentrations of 100 U/mL (approximately 1 nmol/L) or greater and was not blocked by anti-Tac antibody suggest the possibility that this effect of IL2 may be mediated by intermediate-affinity IL2 receptors.

Our studies demonstrated that rIL2 did not stimulate or inhibit BM growth when added directly to BM cultures. IL2 was shown to stimulate BPA production by T cells, LGL, and NK cells, whereas no GM-CSF or CFU-GM–inhibitory activity was generated by these IL2-stimulated cells. We also demonstrated that IL2-stimulated BPA production by these cell populations appeared to be mediated by a non-Tac receptor or by an epitope of the IL2 receptor not recognized by anti-Tac. These data support the hypothesis that cells involved in the immune response, specifically T cells and NK cells, play a role in the regulation of hematopoiesis and that the modulation of hematopoietic growth involves complex interactions between a variety of cell types and their humoral products. Alteration of the number of T cells and NK cells, their state of activation, and their selective production of lymphokines may contribute to hematopoietic abnormalities. Understanding these interactions is important in dissecting the mechanisms of normal hematopoiesis and the derangements of regulation that may occur in various disease states.

REFERENCES

1. Ruscetti FW, Chervenick PA: Release of colony stimulating activity from thymus derived lymphocytes. J Clin Invest 55:520, 1975

2. Howard M, Burgess A, McPhee D, Metcalf D: T-cell hybridoma secreting hemopoietic regulatory molecules: Granulocyte-macrophage and eosinophil colony stimulating factors. Cell 18:993, 1979

3. Golde DW, Bersch N, Quan SG, Lusis AJ: Production of erythroid-potentiating activity by a human T-lymphoblast cell line. Proc Natl Acad Sci USA 77:593, 1980

4. Hansson M, Beran M, Anderson B, Kiessling R: Inhibition of in vitro granulopoiesis by autologous and allogeneic human NK cells. J Immunol 129:126, 1982

5. Mangan KF, Hartnett ME, Matis SA, Winkelstein A, Abo T: Natural killer cells suppress human erythroid stem cell proliferation in vitro. Blood 63:260, 1984

6. Pistoia V, Ghio A, Nocera A, Leprini A, Perata A, Ferrarini M: Large granular lymphocytes have a promoting activity on human peripheral blood erythroid burst–forming units. Blood 65:464, 1985

7. Degliantoni G, Perussia B, Mangoni L, Trinchieri G: Inhibition of bone marrow colony formation by human natural killer cells and by natural killer cell–derived colony-inhibiting activity. J Exp Med 161:1152, 1985

8. Smith KA: T-cell growth factor. Immunol Rev 51:337, 1980

9. Uchiyama T, Broder S, Waldmann TA: A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. J Immunol 126:1393, 1981

10. Leonard WJ, Depper JM, Uchiyama T, Smith KA, Waldmann TA, Greene WC: A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor; partial characterization of the receptor. Nature 300:267, 1982

11. Depper JM, Leonard WJ, Robb RJ, Waldmann TA, Greene WC: Blockade of the interleukin-2 receptor by anti-Tac antibody: Inhibition of human lymphocyte activation. J Immunol 131:690, 1983

12. Reem GH, Yeh NH: Interleukin-2 regulates expression of its receptor and synthesis of gamma interferon by human T lymphocytes. Science 225:429, 1984

13. Palacios R: Production of lymphokines by circulating human T lymphocytes that express or lack receptors for interleukin 2. J Immunol 132:1833, 1984

14. Robb RJ, Munck A, Smith KA: T-cell growth factor receptors: Quantitation, specificity and biological relevance. J Exp Med 154:1455, 1981

15. Robb RJ, Greene WC, Rusk CM: Low and high affinity cellular receptors for interleukin 2: Implications for the level of Tac antigen. J Exp Med 160:1126, 1984

16. Bich-Thuy LT, Fauci AS: Proliferation of human lymphocytes which were not pre-activated in vitro to recombinant interleukin 2. Clin Res 33:372, 1985 (abstr)

17. Lanier LL, Le AM, Phillips JH, Warner NL, Babcock GF: Subpopulations of human natural killer cells defined by expression of the Leu 7 (HNK-1) and Leu 11 (NKP-15) antigens. J Immunol 131:1789, 1983

18. Perussia B, Trinchieri G, Jackson A, Warner NL, Faust J, Rumpold H, Kraft D, Lanier LL: The F_c receptor for IgG on human natural killer cells: Phenotypic, functional and comparative studies using monoclonal antibodies. J Immunol 133:180, 1984

19. Trinchieri G, Matsumoto-Kobayashi M, Clark SC, Seebra J, London L, Perussia B: Response of resting human peripheral blood natural killer cells to interleukin 2. J Exp Med 160:1147, 1984

20. Lanier LL, Benike CJ, Phillips JH, Engleman EG: Recombinant interleukin 2 enhanced natural killer cell–mediated cytotoxicity in human lymphocyte subpopulations expressing the Leu7 and Leu11 antigens. J Immunol 134:794, 1985

21. Shaw ARE, Buckley RC, Merryweather JP, Barr PJ: Modulation of human natural killer cell activity by recombinant human interleukin 2. Cell Immunol 91:193, 1985

22. Mukaida N, Kashahara T, Hosol J, Shiori-Nakano K, Kawai T: Effects of anti-Tac antibody on the response of large granular lymphocytes to interleukin 2. Immunology 57:137, 1986

23. Rosenberg SA, Grimm EA, McGrogan M, Doyle M, Kawasaki E, Koths K, Mark DF: Biological activity of recombinant interleukin-2 produced in Escherichia coli. Science 223:1412, 1984

24. Wang A, Lu S-D, Mark DF: Site specific mutagenesis of the human interleukin-2 gene: Structure-function analyses of the cysteine residues. Science 224:1431, 1984

25. Greenberg PL, Baker S, Link M, Minowada J: Immunologic selection of hemopoietic precursor cells utilizing antibody-mediated plate binding ("panning"). Blood 65:190, 1985

26. Phillips JH, Warner NL, Lanier LL: Correlation of biophysical properties and cell surface antigenic profile of Percoll gradient–separated human natural killer cells. Nat Immun Cell Growth Regul 3:73, 1984

27. Civin CI, Strauss LL, Brovall C, Fackler MJ, Swartz JF, Shaper JH: Antigenic analysis of hemopoiesis III. A hemopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-Ia cells. J Immunol 133:157, 1984

28. Gasson JC, Weisbart RH, DiPersio JF, Golde DW: GM-CSF: Molecular biology, function and clinical response. Exp Hematol 14:418, 1986 (abstr)

29. Estrov Z, Roifman C, Wang Y, Grunberger T, Gelfand EW, Freedman MH: The regulatory role of interleukin 2–responsive T lymphocytes on early and mature erythroid progenitor proliferation. Blood 67:1607, 1986

30. Wright S, Weitzen ML, Kahle R, Granger GA, Bonavida B: Studies on the mechanism of natural killer cytotoxicity II. Coculture of human PBL with NK-sensitive or resistant cell lines stimulates release of natural killer cytotoxic factors (NKCF) selectively cytotoxic to NK-sensitive target cells. J Immunol 130:2479, 1983

31. Tsudo M, Kozak RW, Goldman CK, Waldman TA: Demonstration of a non-Tac peptide that binds interleukin 2: A potential participant in a multichain interleukin 2 receptor complex. Proc Natl Acad Sci USA 83:9694, 1986

32. Robb RJ, Rusk CM, Yodoi J, Greene WC: Interleukin 2 binding molecule distinct from the Tac protein: Analysis of its role in formation of high affinity receptors. Proc Nat Acad Sci USA 84:2002, 1987

33. Dukovich M, Yuji W, Bich-Thuy LT, Katz P, Cullen BR, Kehrl JH, Greene WC: A second human interleukin-2 binding protein that may be a component of high affinity interleukin-2 receptors. Nature 327:518, 1987