Receptor-Specific Inhibition of Bone Marrow Erythropoiesis by Recombinant DNA-Derived Interleukin-2

By Stefan E.G. Burdach and Lee J. Levitt

Interleukin-2 (IL-2) induces differential secretion of lymphokines by IL-2 receptor (IL-2R)-positive and IL-2R-negative T cells. We studied T cell IL-2R-specific modulation of adult bone marrow erythropoiesis by recombinant IL-2 (rIL-2). IL-2R were induced by CD3 T cell surface determinant—triggering and analyzed by cytotoxicity. Bone marrow monocyte and T cell-depleted (NAB-T) target cells were assessed for early erythroid progenitor expression (BFU-E) in the presence of 0 to 10^5 U/mL of rIL-2. rIL-2 had no significant effect on BFU-E expression in the absence of T cells or in the presence of IL-2R-negative T cells. rIL-2 caused a dose-dependent inhibition (75% to 90%) of BFU-E in the presence of autologous IL-2R-positive T cells. The addition of anti-IL2-receptor antibody to cultures containing rIL-2 plus IL-2R-positive T cells entirely abrogated rIL-2-mediated inhibition of BFU-E. In the presence of rIL-2 (10^2 U/mL) production of interferon gamma (IF-gamma) by adult marrow CD3-triggered IL-2R-positive T cells was increased 37- to 125-fold compared to IL-2R-negative T cells. rIF-gamma caused a dose-dependent inhibition (88% ± 17% at 10^5 U/mL) of adult BFU-E in the presence of CD3-triggered autologous T cells. rIL2-mediated inhibition of adult BFU-E in the presence of IL-2R-positive T cells was partially abrogated (52% ± 16%) following addition of monospecific IF-gamma antibody. These results demonstrate (a) rIL-2 modulation of adult marrow erythropoiesis is selectively dependent upon both the presence or absence of autologous T cells and the IL-2R status of these T cells; and (b) rIL-2-induced inhibition of adult marrow erythropoiesis is mediated in part by release of IF-gamma from IL-2R-positive T cells.

Experimental evidence indicates that T lymphocytes and specific T-derived lymphokines play an important and complex role in the regulation of erythropoiesis. The concept that lymphokines as identifiable proteins mediate specific regulatory functions provides a relevant approach for clarifying the physiology of hematopoietic immunoregulation. The availability of human lymphokines produced by recombinant gene technology enables further dissection of the regulatory mechanisms involved in cellular communication between the hematopoietic and immune systems.

Interleukin-2 (IL-2) is a 15-kd glycoprotein produced by helper T lymphocytes in response to both antigen presentation and stimulation with macrophage-derived interleukin-1. IL-2 induces proliferation of both helper/inducer and suppressor/cytotoxic T cells with accompanying lymphokine release. Human IL-2 has been biochemically characterized and molecularly cloned and is known to be encoded by a single gene located on chromosome 4. Recombinant DNA-derived IL-2 (rIL-2) has recently been made available as a purified protein.

It has been established by several investigators that the response of T cells to IL-2 requires expression on the T cell surface of the 55-kd IL-2 receptor. Two monoclonal antibodies recognizing the same determinant have been raised against this IL-2 receptor (IL-2R) and have been termed anti-Tac and 2A3. This IL-2 receptor is induced by antigen presentation to the T cell receptor for antigen (T,R/Ti complex). Presentation of antigen to the T cell antigen receptor can be substituted by binding of anti-Ti (CD3) monoclonal antibody or lectins. Following stimulation with antigen or lectin, T cells primarily express a low-density IL-2R. This receptor has a high affinity for IL-2 and has been thought to be responsible for mediating biologic effects of the lymphokine. In response to IL-2, T lymphocytes increase their IL-2R expression, express a low-affinity IL-2R and release interferon gamma (IF-gamma).

The precise regulatory role of IL-2 in hematopoiesis has not yet been determined. We studied modulation of adult marrow early erythroid progenitor (BFU-E) expression by IL-2R-positive and IL-2R-negative T cells in response to purified recombinant human IL-2 and purified human recombinant IF-gamma. Our results demonstrate that IL-2 exerts a specific regulatory role on erythropoiesis in vitro, that IL-2 modulation of adult marrow erythropoiesis is selectively dependent upon both the presence of autologous T cells and the IL-2 receptor status of those T cells, and that IL-2-induced inhibition of adult early erythroid progenitors in the presence of IL-2R-positive T cells is mediated at least in part by IF-gamma.

Materials and Methods

Recombinant DNA-derived lymphokines. Highly purified human interleukin-2, derived from E coli transfected with human c-DNA, was kindly provided by Drs Kirston Koths and Ed Bradley of Cetus Corporation, Emeryville, CA. Specific activity, as assessed by [3H]thymidine incorporation into IL-2-dependent murine T cell lines, ranged from 4.2 to 6.0 x 10^6 U/g protein. The purity of the preparations was 98% by either sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) densitometry or high-performance liquid chromatography (HPLC). An excipient control

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was used as an IL-2 buffer control and contained all ingredients of the IL-2 formulation except IL-2. Human recombinant DNA-derived IF-gamma was produced in E. coli following gene synthesis and transfection (AMGen, Thousand Oaks, CA). The material was >95% pure by SDS-PAGE densitometry. The endotoxin content of the lymphokine stocks and working solutions was assessed by limulus assay; endotoxin content was at all times negligible (<10^-10 g/mL).

**Cells and cell separation procedures.** All studies were approved by the Stanford University Human Subjects Experimentation Committee. Human bone marrow was obtained from normal adult donors. The low density (buoyant) mononuclear cell fraction was depleted of monocytes by two consecutive plastic adherence procedures as previously described. Residual monocyte contamination of depleted of monocytes by two consecutive plastic adherence procedures as previously described. Residual monocyte contamination of the lymphokine stocks and working solutions was assessed by indirect immunofluorescence with monoclonal antibody Leu M3 and FITC-conjugated purified goat anti-mouse polyclonal IgG and was <1%. The adherent fraction was recovered at 4°C from the first adherence procedure. Indirect immunofluorescence determinations of adherent fraction yielded 26% to 77% of the cells positive with monocytic monoclonal antibody Leu M3, 12% to 34% positive with pan-B lymphocyte CD20 (B1, Coulter, Hialeah, FL) monoclonal antibody, and less than 1% positive with either pan-T lymphocyte CD3 (IgG, Leu 4) or pan-natural killer lymphocyte CD16 (Leu 11c) monoclonal antibodies.

The nonadherent buoyant (NAB) mononuclear cell fraction was further depleted of T cells by solid-phase indirect immunoadsorption as previously described. Nonbound (NAB-T) and bound (T) cells were recovered by differential elution at 4°C and 23°C, respectively. Residual T lymphocyte contamination of NAB-T cells as assessed by indirect immunofluorescence following simultaneous incubation with anti-CD5 (Leu 1, provided by Dr R. Levy, Stanford University), anti-CD8 (Leu 2), and anti-CD4 (Leu 3) antibodies was less than 1%. Purity of the bound (T) fraction recovered from the first-step immunoadsorption procedure was 95% ± 3% as assessed by a three-stage immunofluorescence assay employing anti-CD3 IgG (Leu 4), goat antimouse IgG, and rabbit antigoat IgG-FITC (fluorescein isothiocyanate conjugate).

**Induction of IL-2 receptor.** Bone marrow mononuclear cells were depleted of monocytes to blunt endogenous IL-1 and subsequent IL-2 production. Abrogation of endogenous IL-2 production was monitored by IL-2 bioassay. Nonadherent cells were incubated with CD3 monoclonal antibody, (OKT3 IgG, 1.25 x 10^7 g/10^6 cells), which recognizes a determinant (CD3) on the T cell surface associated with the T cell receptor for antigen. Control cells were incubated with Leu 1 monoclonal antibody, which recognizes a determinant (CD5) distinct from the antigen receptor. CD3-triggered and CD5-triggered cells were cultured in 15% fetal calf serum in the presence or absence of 10^2 U/mL IL-2. At time 0 and after 1, 2, 4, 5, and 6 days, cell samples from the four culture conditions (CD3 or CD5 incubations ± rIL-2) were stained with OKT3-FITC and anti-IL-2 receptor A23 (IgG, Becton Dickinson, Mountain View, CA), phycoerythrin antibodies. Lymphocyte-gated cells (10^6 cells per sample) were analyzed with two-color flow cytometry on an Ortho (Raritan, NJ) fluorogoraph 1250.

**Functional assessment of IL-2 receptor blockade by anti-IL-2 receptor monoclonal antibody.** Bone marrow T cells were recovered from the bound fraction following indirect solid-phase immunoadsorption with CD3 mononuclear antibody and incubated with anti-CD3 (OKT3) monoclonal antibody (1.25 x 10^-7 g/10^6 T cells) to induce surface expression of the IL-2R. Control T cells were incubated with anti-CD5 antibody. Both T cell populations, IL-2 receptor-positive and IL-2 receptor-negative, were cultured in methylcellulose in duplicate in the absence or presence of various concentrations of recombinant IL-2 (10^3 to 10^5 U/mL). Culture conditions were identical to those of the clonal erythroid progenitor assay. T cell proliferation at day 14 was assessed by enumeration of three to five fields at 10x magnification, employing an inverted microscope and a microcytometer. T cell proliferation was also assessed in the presence and absence of various concentrations of anti-IL-2 receptor monoclonal antibody.

Recombinant IL-2 caused a consistent dose-dependent proliferation of CD3-triggered T cells in methylcellulose culture. CD3-stimulated T cells increased from 43.5 ± 16.5 per three microcytometer fields in the absence of rIL-2 to 457.5 ± 2.5 in the presence of 10^3 U/mL recombinant IL-2 after 12 days in culture. In contrast, CD3-triggered adult marrow T cells were 46.5 ± 12.2 after 12 days of culture in the presence of 10^3 U/mL rIL-2. Anti-IL-2 receptor antibody (Tac-1, IgG4, provided by Dr T. Waldmann, NIH, Bethesda, MD) caused a consistent dose-dependent inhibition of recombinant IL-2 (10^5 U/mL)-induced T cell proliferation with complete abrogation at 10^-8 g/mL antibody.

**Assessment of IF-gamma release in response to rIL-2.** IF-gamma was assessed by solid-phase radioimmunoassay (Centocor, Malvern, PA) in supernatants of CD5- or CD3-triggered T cells—containing marrow NAB cells (10^6/mL) cultured in the presence and absence of 10^3 U/mL rIL-2. T cell content of NAB marrow fractions was assessed by indirect immunofluorescence with pan-T Leu 4 monoclonal antibody. IF-gamma secretion in response to 10^5 U/mL rIL-2 was also assessed in the supernatants of purified CD3-triggered cultured marrow T cells (10^4 to 10^6/mL). IF-gamma concentrates were assessed following three to nine days of target cell incubation at 37°C.

**Clonal progenitor assay.** Bone marrow monocyte and T cell-depleted mononuclear target cells (NAB-T—enriched progenitors) were cultured alone or cocultured with CD3-triggered autologous T cells. A selected lot of Mo conditioned medium (derived from a HTLV-II–infected T lymphoblast line, kindly provided by Drs J. Gasson and D. Golde, UCLA) was tested for IF-gamma and IL-2 content was employed as a source of exogenous burst-promoting activity (BPA) and was added at a final concentration of 1%. The final concentration of IF-gamma and IL-2 in culture due to the addition of Mo-conditioned medium were <0.1 U/mL. Cultures containing 0.25 U/mL purified human urinary erythropoietin (containing no detectable BPA; specific activity >10^9 U/mg; Terry Fox Laboratories, Vancouver, BC) and 15% fetal calf serum, Iscove's modified Dulbecco's medium, and 1.1% methylcellulose with and without exogenous BPA were established at a volume of 0.25 mL in cluster plates. Clonogenic progenitors giving rise to erythroid bursts (BFU-E) were enumerated at days 12 to 14 as previously described. In control experiments serum concentrations from 0% to 30% and bovine albumin concentrations from 0% to 1% were studied in the absence and presence of exogenous erythroid burst-promoting activity to facilitate detection of BPA. In the presence of 15% serum and no additional albumin the addition of 1% Mo conditioned medium increased BFU-E expression from 0 to 179 ± 28 BFU-E per 1 x 10^5 NAB-T marrow cells (<0.01). Higher concentrations of Mo-conditioned medium did not provide additional BPA.

Marrow target cells were cultured at 1.5 x 10^5/mL as derived from experiments assessing linearity of clonogenicity over varying target cell concentrations under optimal concentrations of BPA. The ratio for NAB-T:T was 1:0.2. Adherent cells were also cocultured at an NAB-T:adherent cell ratio of 1:0.2.

**Experimental design to assess lymphokine modulation of erythroid progenitor expression.** Erythropoietic progenitor expression in the presence of 0 to 10^5 U/mL recombinant DNA-derived IL-2 was studied in the absence of T lymphocytes, in the presence of CD3-triggered (IL-2 receptor-positive) autologous T cells, and in the presence of control CD5-triggered (IL-2 receptor-negative) autologous T cells to assess T cell and IL-2 receptor-dependent and independent modulation of progenitor expression by IL-2. Progeni-
Antibody concentration of up to 1 log, relative to suspension culture, synthetic or natural IF-gamma as assessed by solid-phase radiimunonosorbent assay. Student's t-test was employed in these studies (Meloy, Springfield, VA). In suspension against natural IF-gamma and the recombinant IF-gamma preparation utilized in these studies, 6.6 x 10^5 of this antibody was capable of neutralizing 1 unit of IF-gamma from IL-2 receptor-positive T cells in response to rIL-2. For IL-2 receptor-positive T cells and anti-IF-gamma antibody but without recombinant IL-2. The results of control experiments, in which we assessed cell proliferation under culture conditions identical to those in our clonal progenitor assay, indicated that the IL-2 receptor antibody effectively blocked rIL-2-mediated proliferation of IL-2 receptor-positive T cells and also indicated the concentration of antibody required to block the effects of rIL-2 on IL-2 receptor-positive T cells.

To assess the effects of IF-gamma on adult erythropoiesis, adult marrow progenitor expression under concentrations of recombinant DNA-derived IF-gamma ranging from 0 to 10 U/mL was studied in the absence of monocytes and T cells, in the presence of autologous non-CD3-stimulated T cells and adherent cells, and in the presence of autologous CD3-stimulated T cells alone. Progenitor expression in the presence of IF-gamma under each of these four coculture conditions was compared to an internal control containing the identical autologous accessory cell and target cell populations plus the recombinant IF-gamma diluent. These experiments were designed to elucidate the contribution of T cells, CD5-triggered cells, and the triggering of the T cell antigen receptor in modulating IF-gamma effects on erythropoiesis.

Finally, we asked whether the effects of IL-2, on erythroid progenitor growth in the presence of autologous IL-2 receptor-positive T cells, are mediated either directly or indirectly by release of IF-gamma from IL-2R-positive T cells in response to rIL-2. For this purpose, erythroid progenitor expression was studied in the presence of both recombinant interleukin-2 and autologous interleukin-2 receptor-positive T cells, and in the absence or presence of various concentrations of anti-IF-gamma monoclonal antibody. These results were compared to internal controls containing IL-2 receptor-positive T cells and anti-IF-gamma antibody but without recombinant IL-2.

Anti-IF-gamma monoclonal antibody 45/6 (IgG1) was pretested against natural IF-gamma and the recombinant IF-gamma preparation utilized in these studies (Meloy, Springfield, VA). In suspension 6.6 x 10^6 of this antibody was capable of neutralizing 1 unit of synthetic or natural IF-gamma as assessed by solid-phase radiimunonosorbent assay. In semisolid medium (methylocellose) an increase of antibody concentration of up to 1 log, relative to suspension culture, was required for neutralization. The IF-gamma antibody did not cross-react with human alpha and beta interferons.

All coculture experiments were performed with autologous mononuclear cells.

Statistics. Results are expressed as mean ± standard error of the mean unless otherwise indicated. The two-sample unpaired Student's t-test and, when appropriate, the Student's t-test for paired observations were used in statistical analysis. All probability values for testing inhibition or stimulation were determined for a one-tailed distribution.

RESULTS

Induction of IL-2 receptor. Figure 1 depicts the kinetics of IL-2 receptor expression on adult bone marrow lymphocytes. In the presence of CD3-triggering antibody, lymphocytes continue to express IL-2 receptors for at least 1 week. Without CD3-triggering, no significant IL-2 receptor expression was observed. The addition of 10 U/mL rIL-2 to CD3-activated but not to CD5-activated T lymphocytes significantly increased both the percentage and the fluorescence intensity (data not shown) of IL-2 receptor-positive cells (p < 0.01). Since the intensity of fluorescence is proportional to the amount of antibody bound per cell, the increase in IL-2-receptor antibody fluorescence represents increased density of antibody binding sites per cell.

Modulation of early erythroid progenitor expression by rIL-2. Adult marrow erythroid progenitors grown in the presence of Mo conditioned medium were inhibited by rIL-2 only in the presence of autologous CD3-activated marrow T cells (Fig 2). Compared to control cultures grown in the absence of rIL-2 but containing IL-2 receptor-positive T cells (126 ± 32 BFU-E per 1.5 x 10^3 NAB-T), the addition of 10 U/mL rIL-2 caused a 30% decrease, 10^2 U/mL rIL-2 caused an 86% decrease (p < 0.01), and 10^3 U/mL caused a 75% decrease (p < 0.01) in BFU-E (n = 5). rIL-2 had little stimulatory effect on adult marrow BFU-E in the presence of IL-2 receptor-negative marrow T cells (Fig 2). Adult marrow BFU-E could not be grown in the low serum assay employed in these studies in the absence of Mo conditioned medium. The addition of IL-2 receptor-negative cells plus rIL-2 did not induce BFU-E expression in the absence of Mo conditioned medium (n = 3). Serum albumin was added to cultures in subsequent experiments to overcome these growth restraints (n = 4). Under these conditions and in the absence
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Reversal of rIL-2-mediated inhibition of erythropoiesis by anti–IL-2 receptor antibody. Inhibition of BFU-E by rIL-2 in the presence of IL-2R-positive T cells was reversed by anti–IL-2 receptor antibody in a dose-dependent manner (Fig 3). Marrow erythroid progenitors, grown in the presence of 10^5 U/mL rIL-2 and autologous IL-2 receptor-positive T cells, increased from 8% ± 8% of control BFU-E in the absence of anti–IL-2 receptor antibody to 101% ± 27% of control BFU-E at 10^7 ng/mL anti–IL-2 receptor antibody (n = 3).

**rIL-2-mediated secretion of IF-gamma.** We studied IF-gamma production by CD3-triggered (IL-2R-positive) and CD5-triggered (IL-2R-negative) purified marrow T cells, and by marrow NAB cells containing IL-2R-positive or IL-2R-negative T cells. IF-gamma release in the presence of 10^5 U/mL rIL-2 by 10^6/mL marrow NAB cells containing IL-2R-positive T cells ranged between 190 and 420 U/mL. In contrast, IF-gamma release by 10^6/mL marrow NAB cells containing IL-2R-negative T cells was only 1.5 to 11.5 U/mL in the presence of 10^5 U/mL rIL-2 and was 0.2 to 0.5 U/mL in the absence of rIL-2. When purified marrow IL-2R-negative T cells were assessed separately, IF-gamma production in the presence of 10^5 U/mL rIL-2 was 17 to 50 U/mL at 10^5 T cells per mL and 330 to 480 U/mL at 10^6 T cells per mL.

Inhibition of early erythroid progenitor expression by recombinant IF-gamma. Since IL-2 receptor–positive T cells release IF-gamma in response to IL-2, we studied the effects of IF-gamma on erythropoiesis and assessed its interaction with rIL-2 in our culture systems. Significant inhibition of marrow erythropoiesis by recombinant IF-gamma (rIF-gamma) required the presence of either CD3-triggered T cells alone or both CD3 nontriggered T cells plus autologous adherent cells (Table 1). Compared to control cultures grown in the absence of rIF-gamma but containing CD3-triggered T cells without monocytes (160 ± 26 BFU-E per 1.5 × 10^5 NAB-T target cells), the addition of 10^6 U/mL rIF-gamma caused a 48% decrease in BFU-E and the addition of 10^5 U/mL caused an 83% decrease (p < 0.01) (n = 3). In contrast, only minimal inhibition (12% to 20%) of BFU-E by 10^2 to 10^5 U/mL rIF-gamma was observed in the presence of CD5-triggered T cells alone (n = 3). However, if both CD5-triggered T cells plus autologous adherent cells were present in culture, 10^5 U/mL rIF-gamma caused a 47% decrease and 10^4 U/mL caused a 66% decrease in BFU-E (p < 0.01). No significant inhibition of BFU-E by rIF-gamma was demonstrable in the absence of both monocytes.

**Fig 2.** Effect of rIL-2 on early erythroid progenitor expression (BFU-E) from nonadherent buoyant T-depleted (NAB-T) adult bone marrow cells cultured in the presence of Mo-conditioned medium as a source of exogenous BPA, and in the absence or presence of CD5-triggered (IL-2R-neg) and CD3-triggered (IL-2R-pos) T cells. Abscissa: final concentration of rIL-2 in culture. Ordinate: difference in absolute BFU-E numbers between rIL-2-containing cultures and control cultures. NAB-T cells were cultured at 1.5 × 10^5/mL. The ratio of NAB-T:T was 1:0.2. All BFU-E are expressed per 1.5 × 10^5 NAB-T. BFU-E controls and probability values for inhibition or stimulation were: 169 ± 63 (NAB-T, n = 5), 185 ± 65 (NAB-T plus T_M, n = 4), 126 ± 31 (NAB-T plus T_M, n = 5); p < 0.01 at 10^2 and 10^3 U/mL rIL-2. Values represent mean ± SEM from four to five separate experiments.
sis was entirely abrogated by the simultaneous or prior rIF-gamma-mediated inhibition of erythropoiesis in addition to culture of 6.6 x 10^6/mL anti-IF-gamma antibody (data not shown).

± dose-dependent increase from 6% CD3-triggered T cells and 102 U/mL rIL-2 showed a significant increase in BFU-E expression. rIL-2-mediated inhibition of BFU-E is mediated by release of IF-gamma from autologous IL-2 receptor-positive T cells. The assessment of possible differential regulatory effects of rIL-2, utilizing IL-2 receptor-positive and IL-2 receptor-negative T cells, optimally requires an assay system in which endogenous IL-2 production is blunted and in which IL-2 receptor expression can be induced independently of IL-2 production. To abrogate endogenous IL-1 production, we depleted our target mononuclear cells of monocytes by sequential adherence, yielding less than 1% monocyte contamination in the adherence-depleted fractions. Following monocyte depletion, endogenous IL-2 production (assessed by IL-2 bioassay) was blocked and T cell proliferation (as assessed by both prolifer-

and T cells. rIF-gamma-mediated inhibition of erythropoiesis was entirely abrogated by the simultaneous or prior addition to culture of 6.6 x 10^{-5} g/mL anti-IF-gamma antibody (data not shown).

Abrogation of rIL-2-mediated inhibition of BFU-E by IF-gamma antibody. We next asked whether rIL-2-mediated inhibition of BFU-E is mediated by release of IF-gamma from autologous IL-2 receptor-positive T cells. BFU-E expression was assessed in the presence of rIL-2, IL-2 receptor-positive T cells, and anti-IF-gamma antibody (0 to 6.6 x 10^{-5} g/mL) (Fig 4). BFU-E in the presence of CD3-triggered T cells and 10^2 U/mL rIL-2 showed a dose-dependent increase from 6% ± 5% of control (180 ± 25 BFU-E) in the absence of anti-IF-gamma antibody to 52% ± 16% of control at 6.6 x 10^{-5} g/mL anti-IF-gamma antibody (p < 0.01) (n = 3). Control cultures in this set of experiments contained the same autologous CD3-triggered T cells plus the appropriate concentration of anti-IF-gamma antibody but no rIL-2. No substantial further augmentation in BFU-E was observed with concentrations of anti-IF-gamma antibody greater than 6.6 x 10^{-5} g/mL. BFU-E reconstituted in the presence of autologous CD3-triggered T cells plus rIL-2 and anti-IF-gamma antibody were smaller in size and less well hemoglobinized than control marrow BFU-E grown in the absence of IL-2.

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Table 1. Effect of rIF-Gamma on Adult Bone Marrow Erythropoiesis

Data from a representative experiment: Early erythroid progenitors (BFU-E) were scored at day 14. Mo-conditioned medium was used as a source of exogenous BPA in all experiments. Nonadherent buoyant T-depleted target cells (NAB-T) were cultured in duplicate in the absence or presence of either CD3-triggered or CD5-triggered T cells alone or in the presence of CD5-triggered T cells plus adherent cells. The ratio NAB-T:T was 1:0.2 and the final concentration of NAB-T in culture was 1.5 x 10^6 per mL. BFU-E expressed as mean ± SEM from three separate experiments. Control BFU-E: 180 ± 25 per 1.5 x 10^6 NAB-T/mL in the absence of rIL-2 and the presence of CD3-triggered T cells plus anti-IF-gamma antibody.

DISCUSSION

Our results demonstrate that (a) IL-2 receptors can be expressed on adult marrow nonadherent mononuclear cells by triggering the CD3 determinant on the T cell surface associated with the T cell receptor for antigen; (b) IL-2 does not significantly alter adult erythroid progenitor expression of IL-2 receptor-positive T cells; (c) in the presence of CD3-triggered (IL-2-positive) autologous T cells, recombinant DNA-derived IL-2 is a potent dose-dependent inhibitor of human adult in vitro erythropoiesis; (d) blocking of the T cell IL-2 receptor abrogates the effects of IL-2 that are observed in the presence of IL-2 receptor-positive T cells; and (e) IL-2-induced inhibition of marrow erythropoiesis in the presence of IL-2 receptor-positive T cells is mediated, at least in part, by IF-gamma.
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ation in culture and by flow cytometric blast analysis of cell size and granularity [data not shown]), could be detected only following the addition of exogenous IL-2. Utilizing these assay conditions we have demonstrated an unequivocal increase in IL-2 receptor-expressing bone marrow lymphocytes by triggering a T cell antigen receptor-associated surface determinant with CD3 monoclonal antibody. These findings demonstrate that IL-2 receptor expression can occur in the absence of IL-2 production.

Previous studies utilizing fresh peripheral blood lymphocytes or peripheral blood-derived (monocyte-free) T cell clones have also suggested that IL-2 production and IL-2 receptor expression may occur independently. In addition, sequential nuclear “run-off” experiments with lectin-activated lymphocyte populations have demonstrated that gene transcription for the IL-2 receptor precedes gene transcription for the ligand, and that IL-2 receptor mRNA can be induced independently of de novo IL-2 protein synthesis. As demonstrated in our studies utilizing marrow lymphocytes and as previously demonstrated by others using adult peripheral blood mononuclear cells, the addition of IL-2 increases expression of its own receptor. This secondarily induced receptor is distributed at high surface density and displays low affinity for IL-2; in contrast, the primarily induced receptor, which is expressed in the absence of IL-2, is distributed at low density on the cell surface and displays high affinity for the IL-2 ligand. These observations, in toto, are most consistent with the hypothesis that expression of the primarily induced high-affinity IL-2 receptor is related to antigen presentation to T cells, whereas physiologic IL-2 production and subsequent T cell proliferation is more closely associated with the presence of (IL-1-producing) monocytes.

Inhibition of BFU-E by rIL-2 in the presence of IL-2 receptor–positive T cells was observed in the presence and absence of exogenous BPA (Mo conditioned medium). The inability of optimal amounts of exogenous BPA to overcome the inhibitory effects of rIL-2 on BFU-E in the presence of IL-2 receptor–positive T cells suggests that inhibition of BPA release is not the mechanism of rIL-2–mediated inhibition of erythroid progenitor expression. In addition, synergy or additive effects between IL-2 and other lymphokines present in Mo conditioned medium cannot account for the observed inhibitory effects of IL-2, since inhibition of erythropoiesis by IL-2 was observed in the absence of Mo conditioned medium.

As we have demonstrated, bone marrow IL-2 receptor–positive T cells release IF-gamma in response to IL-2. IF-gamma can inhibit in vitro erythropoiesis. The role of accessory cells in mediating this effect, however, remains controversial and the role of specific T cell antigen receptor triggering in mediating IF-gamma effects on erythropoiesis has not been previously assessed. We have demonstrated that adult marrow erythroid progenitors are inhibited by IF-gamma in the presence of both monocytes and T lymphocytes. No significant inhibition of erythroid progenitor expression was observed in the absence of both T cells and monocytes. Minimal inhibition of erythropoiesis by IF-gamma was observed with nonactivated autologous T cells in the absence of monocytes. In contrast, significant inhibition of BFU-E by IF-gamma was seen with CD3-triggered T cells in the absence of monocytes. These data suggest that triggering the T cell receptor for antigen obviates the requirement for monocytes during IF-gamma–mediated inhibition of early erythroid progenitors. Little is known about the differential expression of IF-gamma receptors on resting and antigen-triggered T lymphocytes. We hypothesize that triggering of the T cell antigen receptor, either by antigen-presenting cells such as monocytes or by anti-CD3 monoclonal antibody, renders T lymphocytes more responsive to the erythropoietic inhibitory activity of IF-gamma.

Synergy in inhibition of erythropoiesis between IF-gamma and other lymphokines (eg, IF-alpha or tumor necrosis factor–beta) has been demonstrated. Concomitant production of synergizing inhibitors of erythropoiesis (eg, tumor necrosis factor–beta and IF-gamma) by IL-2 receptor–positive T cells in response to IL-2 may account for the difference between the amount of rIL-gamma (10^3 U/mL) required for erythropoietic inhibition in the absence of rIL-2 and the amount of natural IF-gamma (approximately 10^5 U/mL) released by IL-2 receptor–positive T cells in response to rIL-2.

In addition to IF-gamma release by IL-2 receptor–positive T cells in response to rIL-2, we observed that rIL-2–mediated inhibition of erythropoiesis in the presence of IL-2 receptor–positive autologous T cells can be partially abrogated by monospecific anti–IF-gamma antibody. Fifty-two percent of the erythroid inhibition caused by 10^3 U/mL rIL-2 was neutralized by adding an excess of anti–IF-gamma antibody. Our results provide evidence that release of natural IF-gamma, as a consequence of rIL-2 stimulation of IL-2 receptor–positive T cells, plays a role in mediating rIL-2–induced inhibition of adult marrow erythropoiesis. Less than complete restoration of BFU-E colony growth by anti–IF-gamma antibody, however, with respect to both colony number and colony size, suggests that in addition to IF-gamma, either an additional lymphokine or a cellular suppressive mechanism also participates in rIL-2–induced inhibition of erythropoiesis. In addition to IF-gamma, IL-2 receptor–positive T cells produce a variety of other lymphokines following stimulation by IL-2, eg, tumor necrosis factor–beta/lymphotoxin. Tumor necrosis factor–beta has had demonstrable inhibitory activity against in vitro erythropoiesis in preliminary studies.

In addition to a regulatory role in normal erythropoiesis, activated T cells and T cell–derived lymphokines have been casually implicated in various instances of bone marrow failure. IF-gamma, in particular, has been associated with the pathogenesis of aplastic anemia and with the pathogenesis of red cell aplasia in the T-gamma lymphoproliferative disease syndrome. A sustained pancytopenia has also been observed following IF-gamma treatment of a well-differentiated lymphocytic lymphoma. Our data suggest that in addition to its effect on IF-gamma release, IL-2 in the presence of antigen-triggered T cells may directly or indi-
Rectly exert a separate inhibitory effect on erythropoiesis. IL-2 production, following lectin stimulation of in vitro T cells, was increased 100% to 900% over normal controls in 12 of 17 patients with aplastic anemia. In the same study, 11 of 15 patients with aplastic anemia possessed circulating T cells bearing the IL-2 receptor, whereas circulating T cells derived from multiply transfused control patients did not express the IL-2R in the absence of exogenous lectin stimulation. The role of IL-2 in the pathogenesis of primary and secondary bone marrow failure deserves further study.

Our experiments may also bear on current clinical trials using rIL-2 in the treatment of cancer patients and patients with the acquired immunodeficiency syndrome. Infusion of rIL-2 in human subjects for 2 to 3 weeks in one study was associated with severe anemia requiring transfusion in 24 of 25 cases. No evidence for accelerated red blood cell destruction was observed in this study. Treatment with rIL-2 has been associated with an increase in serum levels of IF-gamma following both continuous infusions and bolus injections of rIL-2. Activated IL-2 receptor-bearing T cells, constituting up to 25% of circulating peripheral blood mononuclear cells, have also been observed in patients following infusion of rIL-2 and may in part mediate the observed inhibitory effects of rIL-2 on in vivo erythropoiesis. The effects of rIL-2 we have observed on in vitro erythropoiesis suggest that the anemia observed in patients treated with rIL-2 may be due, at least in part, to a specific biological effect of IL-2 on red cell production.

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