Regulation of In Vitro Erythropoiesis by Normal T Cells: Evidence for Two T-Cell Subsets With Opposing Function

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Cellular interactions responsible for regulating in vitro erythropoiesis were studied using murine monoclonal antibodies recognizing antigens expressed by human mononuclear cells. Cell populations of interest were negatively selected by complement-dependent cytotoxicity and then evaluated for their effect on in vitro growth of erythroid burst-forming units (BFU-E). The data suggest that normal peripheral blood T cells contain at least two functionally distinct subpopulations with opposing regulatory effects: one that enhances burst formation and one that limits burst formation. Whether these effects are mediated by direct interactions of T cells with BFU-E or with auxiliary cells remains to be determined.

The development of in vitro colony assays for hematopoietic precursors has provided opportunities for analyzing the effects of a variety of cell populations and factors on the growth of committed stem cells. Several studies using these techniques have indicated that lymphocytes, presumably T cells, can enhance in vitro growth of human, canine, and mouse erythroid colonies in vitro. A possible physiologic relevance for these observations has been suggested by additional studies that indicated that T cells promote erythropoiesis in vivo in mice and enhance marrow engraftment in transplanted dogs. The mechanisms responsible for these phenomena and the characteristics of the cells involved have not been defined.

In the current study, a series of murine monoclonal antibodies specific for differentiation antigens expressed by human mononuclear cells were used to study cell populations that regulate in vitro erythropoiesis. The data indicate that at least two subpopulations of T lymphocytes can be defined by their effects on the growth of peripheral-blood-derived erythroid burst-forming units (BFU-E)—one that functions to enhance growth of BFU-E and a second that inhibits growth of BFU-E.

Materials and Methods

Preparation of Cells

Heparinized whole blood was collected from informed adult volunteers. Blood was diluted 1:1 in RPMI medium 1640 and layered over Ficoll-Hypaque (Lymphoprep, Nyegard Co., Oslo, Norway). After centrifugation for 30 min at 400 g, peripheral blood mononuclear cells (PBMC) retained at the interface were collected and washed 3 times. In some experiments, E-rosetting cells, designated T cells, were separated from nonrosetting cells, designated non-T cells, by rosetting with 2-aminoethyl isothiouronium bromide (AET) treated sheep red blood cells. T cells were retained for use after treatment with hemolytic buffer (NH4Cl Tris lysing buffer).

Monoclonal Antibodies

Antibody 7.28 was produced by immunizing mice with the human leukemic T-cell line CEM. This IgM antibody recognizes a lymphohematopoietic differentiation antigen present on most T cells. In a quantitative complement-dependent cytotoxicity assay, 7.28 ascites fluid caused the lysis of 90% of T cells at a dilution of 1:125, 80% at 1:1000, 50% at 1:3000, and less than 20% at 1:25,000. Antibody 9.3 has been described in detail elsewhere. It is a complement-fixing IgG2a immunoglobulin that reacts with a single polypeptide with a molecular weight of 44,000 daltons. The 9.3 antigen is detected on approximately 50%-75% of peripheral blood T cells, 25%-30% of thymocytes, and on cells from 4 of 6 leukemic T-cell lines tested (CEM, 8402, MOLT-4F, and Jurkat). The 9.3-positive T-cell subset is larger than and includes the subset identified by antibody OK-T4.

Antibody 7.2 has also been described in detail elsewhere. It is a complement-fixing IgG2b antibody specific for a common determinant on the human Ia-like p29,34 bimolecular complex. In titration against peripheral blood lymphocytes, the cytotoxic activity of 7.2 ascites fluid reached a plateau of maximal killing at a dilution of 1:4000. The experiments presented here employed a 1:1000 dilution of ascites fluid. At this dilution, between 2% and 10% of normal T cells were lysed.

Complement-Mediated Cytotoxicity

Cells were suspended at a concentration of 1-4 x 10⁶ in 0.1 ml of tissue culture medium (RPMI 1640) containing 20% fetal calf serum (heat-inactivated). An equal volume of either 9.3, 7.28, or 7.2 ascites fluid, diluted as indicated, was added and the cells incubated at room temperature for 30 min. Pooled, prescreened normal rabbit serum (0.2 ml undiluted) was then added as a source of complement (C'), and the incubation was continued for an additional 60 min. After washing 3 times, cells were resuspended to starting volume in supplemented alpha medium for use in BFU-E assays.

The initial experiments included four control groups: (1) media treatment alone, (2) antibody alone, (3) complement alone, and (4) antibody plus heat-inactivated complement. The addition of anti-
body with or without heat-inactivated complement had no effect on BFU-E growth. Complement treatment alone had variable effects on BFU-E formation, therefore, all subsequent experiments used complement-treated controls.

**BFU-E Assay**

BFU-E were grown from peripheral blood mononuclear cells (PBM). After treatment and washing, cells were resuspended in supplemented alpha medium and plated at a final concentration of 1–4 x 10⁶ cells in 0.1 ml plasma clots. Erythropoietin was added at a final concentration of 2 U/ml (Connaught step III, lots 3031 and 3034). Clots were incubated for 14 days at 37°C in 5% CO₂, then harvested, fixed on glass slides, and stained with benzidine. Clusters of three or more colonies were counted as 1 BFU-E.

**RESULTS**

**Treatment of Peripheral Blood Mononuclear Cells With Antibody 9.3 and C’**

The number of BFU-E grown from PBM was significantly reduced by treatment with antibody 9.3 and C’. This effect was diminished when the antibody was diluted 1:25,000 and was completely abrogated at dilutions ≥ 1:125,000 (Fig. 1). These results could be interpreted to suggest either that some BFU-E express the 9.3 antigen or that cells which function to enhance BFU-E growth express 9.3 antigen.

In four subsequent experiments, unfractionated PBM and non-T cells were treated with antibody 9.3 (1:1000) and C’ and then assayed for BFU-E growth. As shown in Fig. 2A, the mean reduction in BFU-E growth from unfractionated PBM was 30% ± 11% (p < 0.05). In contrast, no reduction in BFU-E growth was observed in the T-cell-depleted PBM population (Fig. 2B). These data suggested that the effect of antibody 9.3 and C’ on BFU-E growth from unfractionated PBM cells was due to lysis of T cells that function to enhance BFU-E growth.

**Treatment of Peripheral Blood Mononuclear Cells With Antibody 7.28 and C’**

Treatment of PBM with antibody 7.28 and C’ had no effect on BFU-E growth at high concentrations of antibody (Fig. 1). When lower concentrations of antibody 7.28 were used, however, there was a significant increase in burst formation (p < 0.05). We hypothesized that this effect was due to the elimination of a small proportion of 7.28-positive cells that function to limit BFU-E growth. This hypothesis was supported by the results of six subsequent experiments in which both unfractionated PBM and non-T-cells were treated with antibody 7.28 (1:25,000) and C’ and then assayed for BFU-E growth. As shown in Fig. 2A, the average increase in the number of BFU-E obtained from treated unfractionated PBM was 80% ± 14% (p < 0.01). This effect was not seen, however, when T-cell-depleted PBM were used as the source of BFU-E (Fig. 2B), indicating that the effect observed was mediated by a subset of 7.28-positive T cells.

**Treatment of Normal T Cells With Antibodies 9.3, 7.28, or 7.2 and C’**

The effect of antibody and C’ treatment on the ability of normal T cells to enhance BFU-E growth was determined in coculture studies using untreated non-T-cells as a source of BFU-E. In these experiments, non-T-cells were cultured alone or in the presence of T cells treated either with C’ only or with antibody and C’. In all experiments the T-cell:non-T-cell ratio was 2:1. In five experiments, C’-treated T cells increased burst formation to 180% ± 16% of that obtained without T cells. T cells treated with antibody 9.3 (1:1000) and C’ showed a significant reduction (p < 0.05) in stimulating ability compared to control T cells (Fig. 3). In contrast, T cells treated with antibody 7.28 or 7.2 and C’ showed a significant increase in stimulating ability (p < 0.01).

**DISCUSSION**

Several investigators have studied the effects of adding normal human T cells to cultures of autologous peripheral-blood-derived BFU-E. Their observations...
T CELL SUBSETS REGULATE BFU-E GROWTH

Fig. 2. The effect of antibody (9.3, 7.28, or 7.2) and complement treatment on BFU-E growth from either unfractionated peripheral blood mononuclear cells (A) or from T-cell-depleted mononuclear cells (B). The data are shown as percent of BFU-E growth from control cells treated with complement only. Each bar represents the mean ± SE of normalized data from 4-6 experiments. Control BFU-E growth in different experiments ranged from 15 ± 1.7 to 59 ± 2.5/10^5 cells.

range from detecting no effects to demonstrating an obligatory helper function. These different observations may be explained in part by the different procedures used to prepare subpopulations of cells. Our studies suggest that some peripheral-blood-derived BFU-E can grow without T cells or media specifically conditioned by T cells. However, since the serum used in our plasma clot cultures was screened for the ability to support burst formation, it is possible our culture system contained burst-promoting factors. Nevertheless, our data indicate that the addition of T cells increases the number of detectable BFU-E. This net increase, however, is the result of more complex interactions involving at least two functionally distinct subpopulations with apparently opposing effects.

The number of BFU-E grown from PBM treated with antibody 9.3 and C' was reduced compared to C'-treated controls. Subsequent studies indicated that this reduction was not due to a direct lytic effect of antibody and C' on the BFU-E, but rather to the elimination of T cells that function to augment BFU-E growth in vitro. This point was demonstrated in two ways. First, BFU-E growth from T-cell-depleted PBM was not affected by treatment with antibody and C'. Second, cocultures of treated T cells with untreated non-T PBM indicated that the ability of T cells to stimulate growth was reduced after treatment with 9.3 and C'. The results of these studies indicate that a subset of normal T cells function to increase in vitro burst formation and that some of these cells reside in the subpopulation that expresses antigen 9.3. Whether 9.3-positive cells interact directly with the BFU-E to enhance growth or whether they stimulate auxiliary cells to produce enhancing factors remains to be determined. Additional studies not presented here indicate that 9.3-positive T cells also provide helper/inducer function for B cells in a plaque-forming assay.

PBM treated with antibody 7.28 and C' showed an increase in the number of BFU-E compared to C'-treated controls. This increase in BFU-E growth did not appear to be due to the direct effect of 7.28 and C' on the BFU-E but rather to the elimination of a T-cell population that functions in some way to limit BFU-E growth. This hypothesis was supported in several ways. First, 7.28 and C' treatment had no effect on BFU-E growth from PBM that had been T-cell depleted. Second, coculture studies indicated that the stimulating ability of purified T cells was significantly increased after treatment with 7.28 and C'.

Incubating PBM with 7.2 antibody and C' reduced burst formation by greater than 80%. Since earlier

Fig. 3. The effect of antibody (9.3, 7.28, 7.2) and complement treatment on the ability of T cells to stimulate BFU-E growth is shown as percent of control BFU-E growth. Each bar represents the mean ± SE of data from five experiments. Control BFU-E were grown without T cells. The number of BFU-E grown in control groups from five separate experiments ranged from 9.3 ± 1.9 to 56 ± 4.2/10^5 non-T PBM.
studies have indicated that BFU-E express Ia, this reduction is probably the direct effect of C-mediated cytolyis of the BFU-E.\textsuperscript{13-16} In addition, our data suggest that a population of Ia-positive T cells functions to limit burst formation, since the stimulating ability of T cells was significantly increased after treatment with 7.2 antibody and C. The fact that both Ia and 7.28 antigen are expressed by T cells that limit BFU-E growth suggests either that this functional subset of cells expresses both antigens or that several distinct subsets must interact to mediate this function. Exactly how Ia-positive and 7.28-positive cells limit BFU-E growth, whether this is a direct or indirect effect, remains to be determined. The fact that BFU-E, and T cells that function to limit their growth, express Ia antigens makes it attractive to speculate on the role Ia antigens might play in the recognition or regulation of cell–cell interactions during hematopoietic differentiation.

The results of these studies demonstrate that at least two subpopulations of T lymphocytes can be defined by their effect on the growth of peripheral-blood-derived BFU-E: one population that functions to increase burst formation and a second subpopulation that functions to limit burst formation. Both populations are present in normal peripheral blood T cells, their relative proportions being such that the net effect of adding all T cells to cultures of BFU-E is to increase growth.

**ACKNOWLEDGMENT**

The authors thank Agnes Chang for her expert technical assistance and Sharon Trail for preparing the manuscript.

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