Identification of the Colony-Stimulating Cell in Human Peripheral Blood

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ABSTRACT Bone marrow colony formation in soft gel culture may be stimulated by substances elaborated by human peripheral blood leukocytes. In order to determine the cell type responsible for colony stimulation, peripheral leukocytes were separated by Ficoll-Hypaque gradients and differential glass adhesion. Morphologic, histochemical, and functional criteria were applied to determine the purity of the monocyte, lymphocyte, and neutrophil fractions.

Using these cells as feeder layers and as a source of conditioned medium, evidence was obtained that the monocyte is the colony-stimulating cell of human peripheral blood. Activity greater than that of mixed white cells was obtained with monocyte underlayers, and only monocyte- and macrophage-conditioned media were shown to have significant colony-stimulating activity.

INTRODUCTION

Clonal growth of bone marrow cells in soft gel culture is largely dependent on the availability of stimulating substances (1). Human colony-stimulating factor (CSF) [1] derived from serum and urine has been extensively studied and partially characterized chemically (2). Naturally occurring inhibitors of its action have been identified (3). Since CSF stimulates and supports the growth of bone marrow granulocytes and macrophages in semisolid culture, it has been postulated that CSF may be a humoral mediator of leukopoiesis in vivo (4).

Several lines of evidence indicate that white blood cells themselves may provide an important source of CSF. The stimulation of colony formation by feeder layers of peripheral white cells and conditioned media from leukocyte cultures suggests that leukocytes produce a factor or factors which influence granulocyte and macrophage proliferation in vitro (5–7). Since these data provide a basis for considering autoregulation in the control of leukopoiesis, the identity of the blood cell from which CSF is derived becomes of major significance. In this communication, we present evidence indicating that the monocyte is the colony-stimulating cell of human peripheral blood.

METHODS

Bone marrow obtained from normal volunteers by sternal or iliac puncture was mixed with heparin, sedimented at room temperature, and the cells in the supernatant collected by centrifugation at 150 *g* for 7 min. Red blood cells were lysed (8); the remaining cells were washed twice in complete tissue culture medium and the viable nucleated cell counts were determined with trypan blue.

Normal human monocytes and neutrophils were isolated from peripheral blood utilizing Ficoll-Hypaque gradients and differential glass-adhesiveness according to the method of Boyum (9). As previously described, pure preparations of lymphocytes were separated from leukocyte-rich plasma by passage through a nylon fiber column (10), and macrophages were obtained by growth and differentiation of glass-adherent monocytes cultured in Leighton tubes (11). Fibroblasts were prepared from neonatal human foreskins. The criteria for identification of leukocyte types by morphology in Giemsa-stained preparations, cytochemistry for myeloperoxidase, and functional tests for phagocytosis of bacteria and fungi and for the presence of surface receptors for IgG immunoglobulin on monocytes have been described previously (12). The purity of the different cell populations was: granulocytes > 97%, monocytes > 97%, lymphocytes > 99%. Granulocyte preparations contained rare monocytes and a few nucleated red cells; lymphocytes contained rare myelocytes and degenerating neutrophils.

Isolated leukocytes or fibroblasts were tested for production of CSF by preparing feeder layers at a concentration of 1×10^6 cells in 1 ml of 0.5% agar. Conditioned media were prepared by centrifugation of cultures of either mixed peripheral white cells or pure leukocyte populations (1.5×10^6 cells/ml initial concentration). Conditioned media

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[1] *Abbreviations used in this paper:* CM, conditioned media; CSF, colony-stimulating factor.

TABLE I

Effect of Feeder Layers on Growth of Human Bone Marrow in Agar

Cells in feeder layer	No. of experiments	Colonies per 2×10^5 cells
None	4	1 (0–2)
Mixed blood WBC	4	87 (78–120)
Granulocytes	3	12 (2–24)
Monocytes	4	113 (104–117)
Lymphocytes	3	3 (0–13)
Fibroblasts	3	0

were incorporated in 1 ml agar underlayers in volumes of 0.025–0.2 ml.

Standard techniques were used for bone marrow culture in agar (6). Bone marrow cells (2×10^5) were plated in McCoy's 5A medium supplemented with 15% fetal calf serum and nonessential amino acids in 0.3% agar over a base layer of 0.5% agar. Triplicate plates were incubated in a humidified environment with 7.5% CO_2. Colonies were counted in the upper layer at 10 days using 40 cells as the minimum colony size. Underlayers prepared without CSF or cells were used as controls and uniformly were associated with no growth in the upper agar layer.

RESULTS

Cell feeder layers. The results of four experiments using mixed and pure leukocyte populations as feeder layers are shown in Table I. In each of the experiments, the leukocyte populations and the bone marrow were obtained from a different normal subject. Monocytes consistently had the greatest colony-stimulating effect and in three of four experiments had a greater effect than that observed with mixed white cell feeder layers. Granulocyte preparations generally had less than 10% of the colony-stimulating activity of monocytes but in one experiment had 20%. Lymphocytes cultured with and without phytohemagglutinin and fibroblasts had no detectable colony-stimulating activity.

When granulocytes were mixed with monocytes at a ratio of 1:1 or 2:1, there was no consistent inhibitory or stimulatory effect above that observed with monocytes alone.

Incorporation of 5×10^5 and 1×10^6 monocytes in the feeder layer produced the same number of colonies, suggesting that the system was maximally stimulated under the conditions employed.

Conditioned medium. Conditioned medium from monocytes stimulated bone marrow colony formation in agar. A representative study is shown in Table II. Conditioned media from granulocytes, lymphocytes, and fibroblast cultures were inactive and had no detectable inhibitory effect on medium from monocyte cultures.

The stimulation by media from monocyte cultures was dose dependent in the range of 0.025–0.2 ml added to 1 ml of 0.5% agar underlayer. The lower concentration was equivalent to the 35,000 monocytes per ml of medium.

When conditioned media were obtained from monocyte cultures of various ages, equal potency was observed at days 1, 3, and 4. Supernatants from 6- and 7-day monocyte cultures were about 50% less active (Table II). By the latter time period, transformation to macrophages had occurred, but there were fewer cells left in culture (11). Activity in monocyte-conditioned medium was found to be undiminished after 4 wk's storage at −20°C.

At 7 days of age, agar colonies stimulated both by monocyte underlayers and by conditioned medium contained both granulocytic and mononuclear cells. The mean ratio was 11:1 (0.6% orcein stain).

TABLE II

Effect of Conditioned Media (CM) on Growth of Human Bone Marrow in Agar

Cellular origin of CM	Age of cells in culture	Volume of CM added	Colonies per 2×10^5 cells
	days	*ml*	
None	—	—	2
Monocytes	3	0.1	76
Monocytes	3	0.05	39
Monocytes	3	0.025	8
Monocytes	6	0.1	34
Granulocytes	3	0.1	2
Lymphocytes	3	0.1	0
Fibroblasts	3	0.1	0

DISCUSSION

Evidence that peripheral blood leukocytes produce a factor stimulating granulopoiesis in vitro has been adduced from the efficacy of white cell feeder layer induction of bone marrow colony formation in agar (6, 13). The use of white cell feeder layers is based upon the supposition that humoral factor diffuses from the feeder layer to the culture layer. Support for this concept comes from the finding that cell-free supernatants from cultures of peripheral white cells will also stimulate bone marrow colony formation in agar (5, 7). The colony-stimulating activity of leukocytes is thought to reside in the granule fraction, and inhibition has been demonstrated with N-ethylmalemide but not with cyclohexamide (13).

Haskill, McKnight, and Galbraith (14) and Moore and Williams (15) have used albumin gradient separation in an attempt to identify the cell type producing colony stimulation. These investigators were able to

separate the colony-forming cell from the colony-stimulating cell. High density fractions composed mostly of neutrophils were shown to have no colony-stimulating activity. Moore also found that adherence columns cause retention of colony-stimulating cells, and he postulated that this cell was either a lymphocyte-like cell or a monocyte (15).

We have used a combination of Ficoll-Hypaque gradients and surface-adherent separation techniques to obtain pure populations of peripheral blood leukocytes. Criteria for purity were based on morphology, histochemistry, and function tests. Granulocyte preparations were all phagocytic and had the typical morphology of mature neutrophils and metamyelocytes. Monocytes were weakly peroxidase positive, were phagocytic, and had surface receptors for IgG immunoglobulin (12). Lymphocyte populations were entirely devoid of phagocytic cells, had typical morphology, were peroxidase negative, and were able to undergo blast transformation and production of interferon in response to phytohemagglutinin (10).

Using these cells as feeder layers and as a source of conditioned medium, evidence was obtained that the monocyte is the colony-stimulating cell of human peripheral blood. Activity greater than that of mixed white cells was obtained with monocyte underlayers, and only monocyte- and macrophage-conditioned media were shown to have significant colony-stimulating activity.

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