CONCISE REPORT

Human T Lymphocyte Cell Line Producing Colony-stimulating Activity

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We derived a permanent human T lymphocyte cell line that elaborates a potent colony-stimulating activity (CSA). The line was established with spleen cells from a patient with a T lymphocyte variant of hairy-cell leukemia. These cells form rosettes with sheep erythrocytes, show a proliferative response to phytohemagglutinin, and are lysed by antithymocyte globulin. They do not synthesize immunoglobulin, nor do they contain Epstein-Barr virus. CSA is regularly detected in the supernatant medium after 3 days culture. In the presence of PHA there is augmented elaboration of CSA; maximal activity is reached by 2 days and is 20% greater than that produced by a feeder layer of $1 \times 10^6$ peripheral blood leukocytes. One microliter of the supernatant material stimulated colony formation from the light-density nonadherent fraction of human bone marrow; there was maximal activity between 10 and 50 $\mu $l/ml. Conditioned medium from these cells has little effect in stimulating CFU-C from murine bone marrow. The availability of a human T lymphocyte line producing CSA will provide a source for large quantities of the lymphocyte-derived hormone and permit a definition of factors modulating the interaction of T lymphocytes with granulocyte and monocyte stem cells.

COLONY-STIMULATING ACTIVITY (CSA) is the term given to a group of polypeptide molecules capable of stimulating granulocyte and monocyte colony formation in semisolid gel culture.\(^1\)\(^2\) This specific growth-promoting hormone is believed to be a physiologic granulopoietin and has been isolated from various murine and human tissues.\(^3\)\(^4\) Although murine CSA has been found in a number of tissues,\(^4\) the distribution of human CSA is more limited.\(^3\) Human peripheral blood leukocytes are one of the most potent sources of human CSA, and cells of the monocyte-macrophage complex are the responsible active producers of human CSA.\(^5\)\(^7\) Lymphocytes responding to various mitogens or antigens also elaborate a CSA, suggesting a link between T lymphocyte activation and granulopoietic regulation.\(^8\)\(^9\)\(^10\)

Although human CSA can be obtained from placenta, lung, and peripheral white cells,\(^3\)\(^11\) until recently there has been neither an available homogeneous tissue source for obtaining large quantities of material nor a simple means for studying CSA synthesis. Mouse L cells are an excellent source of murine CSA,\(^12\) and recently two human monocyetic cell lines that produce CSA were described.\(^13\) We have derived a permanent human T lymphocyte cell line that elaborates a potent CSA.
MATERIALS AND METHODS

The cell line (Mo) was established with spleen cells from a patient with a T cell variant of hairy-cell leukemia. A suspension of spleen cells was cultured in T flasks in alpha medium with 20% fetal calf serum and 10^{-4} M a-thioglycerol, and a permanent cell line was obtained. At this writing the cells had been growing continuously for 20 mo. The Mo cells grow in suspension and have a lymphoblastoid morphology. Immunologic characterization of these cells shows them to be of T lymphocyte derivation as evidenced by spontaneous rosette formation with sheep red blood cells and by sensitivity to antithymocyte globulin. Also, these cells do not synthesize immunoglobulin nor do they contain Epstein-Barr virus nuclear antigen (EBNA) or the capsid antigen (VCA). The cell line grows rapidly in serum-supplemented medium and more slowly in serum-free medium. It continuously releases a potent CSA into the supernatant. CSA activity was assayed using the two-layer agar culture technique previously described. Conditioned medium was incorporated into the underlayer, and normal human bone marrow was used as the target cell population in the overlayer. Human marrow was obtained from informed healthy volunteers and used directly after sedimentation or separated on Ficoll-Hypaque gradients and the light-density fraction recovered and depleted of adherent cells. In these fractionated marrow preparations there were no cells producing detectable colony-stimulating or -inhibiting activities. The cultures were incubated at 37°C in a humidified environment of 5% CO2 in air, and colonies of 40 cells or more were counted after 10 days incubation with a dissecting microscope. Determination of colony cell type was ascertained by removing the colonies from the agar with a fine pipette and smearing them on glass slides for cytochemical analysis with peroxidase, α-naphthyl butyrase, and ASD-chloroacetate esterase staining as well as routine Wright-Giemsa stain.

RESULTS

Conditioned medium taken after 3 days subculture regularly contained CSA. Figure 1 shows the dose-response curve for 7-day conditioned medium using normal human bone marrow as a target population in the standard agar colony assay. As little as 1 μl of conditioned medium stimulated more than 20 colonies/10^3 light-density nonadherent human bone marrow cells (p < 0.01). Peak activity was noted at approximately 20 μl/ml, with a plateau to 100 μl. Plateau concentration levels of Mo-conditioned medium stimulated approximately 20% more colonies than a feeder layer of 1 × 10^6 peripheral blood leukocytes. A significant increment in CSA elaborated into the supernatant medium occurred when the Mo cells were cultured with phytohemagglutinin (PHA) (Wellcome, Beckenham, England; 0.01 ml/ml). At submaximally stimulatory concentrations of conditioned medium (5 μl/ml) the effect of PHA was dramatic, as evidenced by the twofold increase in potency. PHA-stimulated Mo-conditioned medium had about 20% more activity than the standard medium at plateau concentrations. The effect of PHA was on the elaboration of CSA by the Mo cells and not on the target cells in the human marrow population. Thus addition of PHA to medium from cells grown in the absence of lectin did not result in increased potency.

Figure 2 shows the time course of CSA elaboration by the cell line. CSA was detected in the conditioned medium by 2 days of culture. The cells could be grown in serum-free medium, and equally potent activity was assayable in the supernate after 9 days of culture. Peak accumulation of CSA did not occur in the serum-free cultures until 1 wk. The addition of PHA resulted in elaboration of one-half maximal activity after 24 hr.

CSA from the Mo line stimulated human myeloid colonies comprised either of neutrophils or of monocytes and macrophages. Eosinophil colonies were
Fig. 1. Effect of various concentrations of Mo cell-conditioned medium on colony formation by normal human bone marrow. Data are from three experiments performed in duplicate. Colony stimulation by a feeder layer of peripheral leukocytes was 42.8 ± 5.9/10⁵ cells for whole marrow and 73.5 ± 8.4/10⁵ for light-density nonadherent fraction determined in seven experiments. ■, Mo-conditioned medium (CM) from PHA-stimulated cultures assayed on light-density nonadherent bone marrow; □, Mo-CM assayed on light-density nonadherent bone marrow; ○, Mo-CM assayed on whole bone marrow.

rarely observed, and no mixed colonies were seen. Conditioned medium from this line has no significant stimulating effect on CFU-C from mouse or rat bone marrow.

Preliminary characterization of the Mo line CSA showed that the active molecule(s) are heat stable at 56°C for 30 min and have an apparent molecular weight of > 100,000 daltons as determined by exclusion by Amicon filters. The active material is retained on concanavalin-A sepharose columns.

**DISCUSSION**

Although there are several murine cell lines that produce CSA in vitro, attempts to find human cell lines producing the mediator have been less successful. Two monocytielike human cell lines producing CSA were recently established, and a human lung cell line was reported to elaborate CSA. To our knowledge, this is the first human T lymphoid line that constitutively produces potent CSA in vitro. We tested supernatants from four other human T cell lines and have found no such activity. Also, we screened ten human B lymphoblastoid cell lines and found no evidence of CSA production.

The availability of a cell line producing human T lymphocyte-derived CSA should permit the isolation of large quantities of this hormonal regulator. Addi-
Fig. 2. Time course of elaboration of CSA by Mo line: 1% BSA was used in cultures grown without FCS. Assays on light-density nonadherent fraction of bone marrow. □, Mo-CM from PHA-stimulated cultures; ○, Mo-CM; △, Mo-CM, cells grown in serum-free medium with 1% BSA.

Additionally, it will allow for studies on the modulation of lymphoid CSA biosynthesis and hopefully shed new light on the interaction of T lymphocytes and hematopoiesis.

REFERENCES

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