Selective in vitro Growth of T Lymphocytes from Normal Human Bone Marrows

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sure of the number of suspended particles per unit volume for a given particle size distribution. To discover how much scattering was due to the sludge alone, we subtracted the averaged profile representing scattering strength per unit volume just outside the sludge dump from each averaged profile obtained along the ship's track within the dump region. For a given transect of a dump region, we noted the value of each resultant profile (of net scattering strength per unit volume due to sludge alone) at a prescribed depth and plotted these values (Fig. 2) as a function of distance through the dump region. Figure 2 shows the scattering strength at a depth of 14 m for three crossings of the line dump.

The progressive displacement of the curves in Fig. 2 along the axis represents the southerly drift of the sludge cloud during the tracking period. The decreasing amplitude and increasing width of the curves can be used to estimate the rate of diffusion of the sludge. We compared these data with those presented by Bowden *et al.* (*6*) in a discussion of diffusion from a continuous source at sea. Bowden *et al.* found that the lateral variance of a dye plume increases according to t^m, where t is time and m varied from 1.2 to 2.7. We found that the lateral variance of the line dump increased according to $t^{1.5}$. It therefore seems reasonable to attempt to calculate the local horizontal diffusivity from this kind of data.

Our results indicate that sewage sludge dumped in the ocean is detectable for several hours with commercially available acoustic equipment that has been modified somewhat. The signal-to-noise ratio is higher during daylight than at night because of the lower biological activity in the water column. The space-time evolution of a sludge dump can be followed if regular traverses of the dump area are made, and the three-dimensional distribution of suspended material can be determined with digital processing. Chemical sampling for research or monitoring purposes can be accurately guided in real time by acoustic tracking because the sludge cloud boundaries are easily discernible from the paper strip chart alone.

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Abstract. *Selective growth of T lymphocytes occurred when unfractionated normal human bone marrow cells were cultured with conditioned medium obtained from phytohemagglutinin-stimulated normal human lymphocytes (Ly-CM). Cultures of up to 90 percent T cells have been maintained for more than 9 months. The T cells exhibited a strict growth dependence upon Ly-CM and were consistently negative for Epstein-Barr viral information.*

Human lymphoblastoid cell lines have been derived from normal bone marrows (*1*); all of these lines express characteristics of bone marrow–derived (B) lymphocytes (*2*) and invariably contain Epstein-Barr virus (EBV) information (*3*). The relatively few cell lines with characteristics of thymus-derived (T) lymphocytes are of neoplastic origin (*4*). The long-term maintenance of murine (*5*) and normal human (*6*) T cells has been reported. Mixed leukocyte cultures consisting of cells from two normal donors have resulted in the growth stimulation of responder T cells up to 9 months upon repeated exposure to allogeneic lymphocytes. We describe here our development of a system in which rapidly proliferating, EBV-negative cultures consisting of more than 90 percent T lymphocytes have been selectively grown from unfractionated normal human bone marrow cell suspensions from several donors. Initiation and long-term maintenance of the lymphocytes is strictly dependent on the continuous presence of conditioned medium from phytohemagglutinin (PHA)-stimulated human blood lymphocytes (Ly-CM).

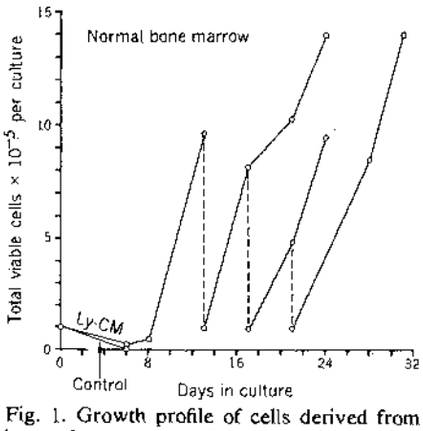

Fig. 1. Growth profile of cells derived from human bone marrow. Growth was induced by PHA-stimulated lymphocyte conditioned medium (Ly-CM). Control cultures are without Ly-CM. The vertical broken lines indicate a culture split.

The Ly-CM was prepared (Associated Biomedic Systems) by culturing pooled normal human lymphocytes. The final concentration of cells was 1×10^6 per milliliter of RMPI (Roswell Park Memorial Institute) medium containing 1 percent autologous serum and 1 percent PHA-M (Difco). After 3 days the medium was collected, concentrated fivefold, dialyzed against ten volumes of 0.15*M* NaCl by ultrafiltration (Amicon), and stored at 4°C until use as the source of Ly-CM. Bone marrow aspirates from normal donors were subjected to hypotonic shock to hemolyze the erythrocytes. The remaining cell pellets were resuspended in 0.15*M* NaCl and distributed into culture tubes (16 by 125 mm; Falcon) at a final concentration of 1×10^5 per milliliter of RPMI 1640 medium containing 20 percent fetal calf serum and 20 percent Ly-CM. The cultures were then incubated at 37°C in 5 percent CO_2 and a humidified atmosphere. Cell growth was monitored at selected intervals by counts of viable cells, at which time morphological staining of cytocentrifuge preparations was routinely done.

The growth curve (Fig. 1) is representative of nine out of nine samples. In both the control cultures, which had received only medium, and the supplemented cultures (Ly-CM) a decline in the viable population to less than 10 percent

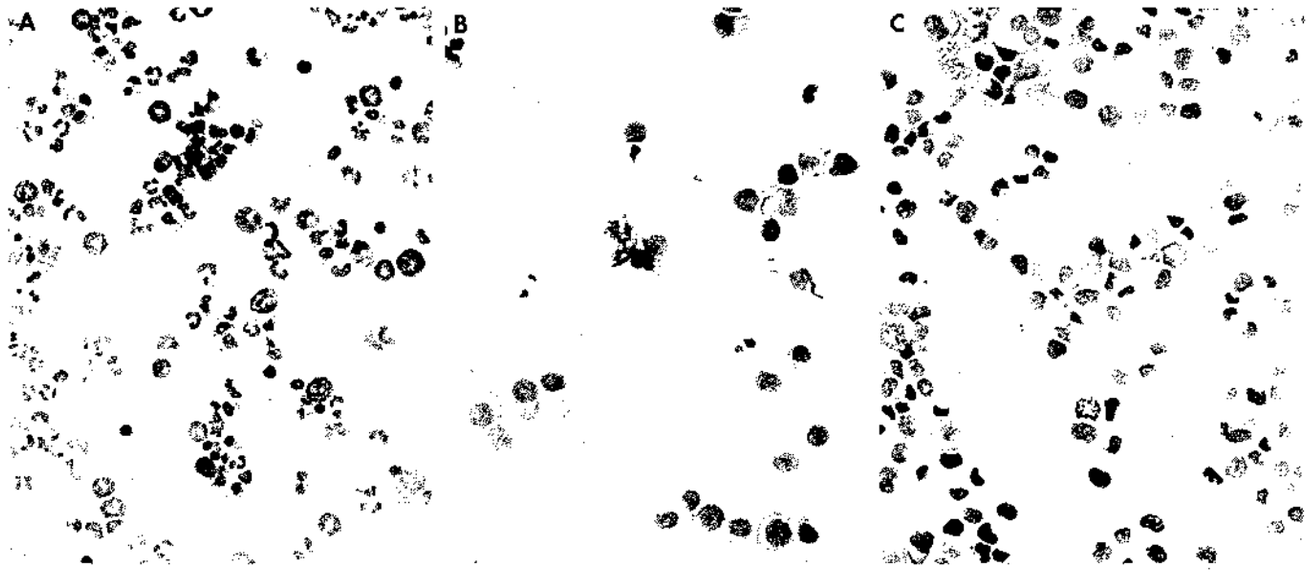

Fig. 2. Cultured cell populations, which had been induced by Ly-CM, from an unfractionated normal bone marrow at (A) day 0, (B) day 8, and (C) day 21.

of the number of cells at the onset of culture ("seed") occurred within the first 6 days. Only the supplemented cultures showed a response by day 8. A tenfold increase over the seed density occurred by day 13, at which time the cultures were split back to 1×10^5 cells per milliliter. Nutrient medium and Ly-CM were replaced. The exponential growth pattern was repeated over the next 7 days and with each subsequent split at days 17 and 21. This continuous pattern of growth has now been maintained for more than 9 months and remains strictly dependent on the Ly-CM.

Morphological examination of the seeded unfractionated bone marrow cells (Fig. 2A) shows predominantly segmented neutrophils and granulocyte precursors with a scarcity of blast cells or lymphocytes that would potentially respond to the Ly-CM. The loss of granulocytes by day 8 (Fig. 2B) would account for the rapid decline of viable cells during the first week in culture. The homogeneous population of cells responding to Ly-CM was responsible for culture regeneration. The characteristic rapid growth rate of these cells is exhibited at day 21 (Fig. 2C) by a mitotic index of 0.033. Assays for phagocytosis of *Candida albicans*, as well as histochemical stains for myeloperoxidase, Sudan black, leukocyte alkaline phosphatase, and naphthol chloroacetate and α-naphthol acetate esterases, were negative. These data suggested that selective growth of either lymphoid cells or undifferentiated blast cells had occurred.

To further delineate the identity of these cells, immunological tests (*7*) were performed. The T and B cell markers, as determined by rosette formation with sheep erythrocytes (E) and complement-bound sensitized erythrocytes (EAC), respectively, were present in 60 to 70 percent of the cells at the end of the second week in culture. After 4 weeks more than 90 percent of the cells demonstrated the T cell marker (E rosettes) but no detectable surface immunoglobulins as determined by immunofluorescence. Additional evidence for the identity of these cells as T cells was provided by culturing a B cell line, Raji, for 3 weeks with the Ly-CM. Only EAC rosettes were observed, indicating that the Ly-CM does not alter the specificity of E rosettes as a T cell marker.

Unlike most B cell lines, these cells have been consistently negative for the Epstein-Barr virus marker, the EBV nuclear antigen (*8*). Another difference from known established lymphoblastoid cell lines was the absolute dependence of the culture growth on the Ly-CM. This was periodically monitored by depriving the cells of the Ly-CM following a culture split; after 3 days the cultures showed a decline in the number of viable cells. In addition, after a 4-hour pulse with 10 μc per culture of [^{3}H]deoxythymidine (specific activity, 12 c/mmole) only 2 percent of the cells demonstrated nuclear grains in the autoradiographic slides as compared to 50 percent of the cells supplemented with Ly-CM.

In summary, we have developed a culture system that selectively induced long-term growth and differentiation of T lymphocyte precursors from unfractionated normal human bone marrows. The results have been reproduced with samples of bone marrow from nine normal donors. The cells were negative for EBV expression based on the absence of detectable EBV nuclear antigen. For continued growth of the cells a factor is required which is present in conditioned medium from PHA-stimulated normal human blood lymphocytes pooled from many donors. The availability of these cells may be relevant to immunological and biochemical characterization of T cells, to the study of control mechanisms for the growth and differentiation of lymphoid cells, and to immunotherapy.

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