Short Communication

ESTABLISHMENT OF MURINE MYELOID-LEUKAEMIA CELL LINE IN SUSPENSION CULTURE

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The vast majority of murine leukaemias are lymphoid in nature. They have been extensively used in chemotherapy studies, and the data obtained have to some extent been applicable to the treatment of myeloid leukaemia in man, but their lymphoid nature has prevented them from being used to study the relationship between myeloid leukaemic cells and normal myeloid stem cells. We have been studying myeloid leukaemia in the RFM mouse (Mori et al., 1977; Preisler et al., 1977) and would like to report the establishment of the leukaemic cells in long-term suspension culture.

RFM mice are bred by brother–sister matings in our breeding laboratory (Mori et al., 1977).

Marrow was obtained from the femurs of RFM mice with myeloid leukaemia (Mori et al., 1977) suspended in RPMI 1640 medium with 10% heat-inactivated foetal calf serum and placed in a 37°C humidified incubator with 5% CO₂. Two days later the cells were resuspended in fresh medium, returned to the incubator and left for 3 weeks. The cells were then recovered by centrifugation and resuspended in either RPMI 1640, McCoy’s 5A, or Dulbecco’s media all with 10% heat-inactivated foetal calf serum.

The cells suspended in McCoy’s 5A or Dulbecco’s media failed to grow. Cells resuspended in RPMI 1640 began to proliferate and were fed twice a week with fresh medium. During attempts to split the cultures it was noticed that the only flasks in which leukaemic cells replicated were those in which a monolayer had developed. Monolayers were prepared from the spleens or marrows of leukaemic or normal RFM mice. All supported the growth of the leukaemic suspension culture. Unconcentrated and 10-fold-concentrated supernatants of these monolayers were tested for in vitro colony-stimulating activity (CSF) using mouse marrow cells as the target, and none was detected. One month later (10–12 passages) growth of the suspension culture cells became independent of the presence of an exogenously supplied monolayer of cells. The cells now grow with cells being present both in the supernatant and as a monolayer attached to the floor of the culture vessel. The cultures can be passaged with either kind of cells.

Morphological and histochemical studies were made as previously described (Mori et al., 1977). Density-cut centrifugation was carried out as previously described, with the exception that Ficol-Hypaque with a density of 1.085 was used. Electron-microscopic studies were kindly provided by Dr Carl Porter (Grant No. CA 13083). Growth in agar used the method of Pluznik and Sachs (see Bradley and Metcalf, 1966). Growth in diffusion chambers in

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vivo was carried out according to the method of Steinberg et al. (1976) modified by the addition of chick embryo extract to ensure clotting within the diffusion chamber. All the above studies were carried out 5 months after establishment of the cell line.

The cell population is heterogeneous with respect to cell size and nuclear morphology. Some nuclei are monocytdoid, others doughnut shaped and still others are round (Fig. 1). Segmented neutrophils have never been observed. Whilst less than 1 cell in 500 is peroxidase positive, using standard histochemical and light-microscope evaluation, using cytofluorographic methods (Salvatori, personal communication) 4-20% of the cells were found to contain peroxidase. All the cells contain N-ASD chloroacetate esterase and the majority are weakly positive for non-specific esterase as well. About 15% are PAS positive, but many of the positive cells appear to be degenerating. The cells are not phagocytic.

Electron-microscope studies demonstrated that the majority of cells have a monocytdoid appearance (Fig. 2a). Virus-like particles can be seen budding into cytoplasmic vacuoles (Fig. 2b).

In suspension culture, the cells grow with a high death rate. Several days after seeding in fresh medium, as many as 20-30% of the cells fail to exclude trypan blue. Density-cut centrifugation using Ficoll-Hypaque with a density of 1.085 can be used to separate living from dead cells. Virtually 100% of the cells above the interface exclude trypan blue, whilst the majority of pelleted cells fail to exclude trypan blue. The addition of murine CSF to the culture medium (final concentration v/v of 10% mouse lung-conditioned medium) failed to alter the growth rate of the cells in culture, and did not induce differentiation (Fig. 3).

The clonogenic potential of the tissue culture cells was determined both in vivo and in vitro. The Table gives the results obtained when the tissue-culture cells

Fig. 1.—Tissue-culture leukaemia cells stained with Wright-Giemsa. (x 30) the small cells with pyknotic nuclei are PAS positive. Cells with doughnut-shaped nuclei lack cytoplasmic granules and staining characteristics of normal murine metamyelocytes.

MURINE MYELOID LEUKAEMIC CELLS IN CULTURE
Fig. 2.—(a) Monocytoid cells (× 4500). (b) Budding of virus-like particles into cytoplasm (× 2700).
were cloned in diffusion chambers in vivo and in vitro. The diffusion chambers provided the best growth conditions. Under these conditions about 1 cell in 1000 produced a colony, some of which consisted of several hundred cells. A few colonies were of the dispersed variety and clearly contained cells which were indistinguishable morphologically from macrophages. Fig. 4 shows that the morphology of the majority of cells was identical to that of the original cells, but some cells appeared to be macrophages. Pre-irradiation of the host animals did not affect the number of colonies. The absolute number of cells in the diffusion chambers increased 10-fold during growth within the chambers, whether or not the animal had been irradiated before implantation of the chamber.

Growth in vitro in the agar system produced 8 colonies/10^5 cells. Some colonies were compact in gross morphology, and consisted of cells which were indistinguishable from those that were originally plated. Other colonies, however, clearly consisted of cells which were indistinguishable from macrophages. The addition of murine colony-stimulating factor had no significant effect upon colony formation.

The malignancy of the cell line was tested 5 months after growth had been established in culture. Inoculation of 10^6 tissue-culture cells into syngeneic RFM/UN mice by a variety of routes produced 100% mortality. Mice inoculated i.v. died 19–25 days after cell inoculation, with splenomegaly of 200–400 mg. I.p. inoculation of tissue-culture cells resulted in death 25–35 days later, with half the mice developing intra-abdominal masses weighing 200–300 mg and splenomegaly of 300–400 mg. S.c. inoculation produced tumours at the site of inoculation weighing (at the time of death) about 200 mg and splenomegaly of 200–300 mg. Inoculation of 10^4 cells was also lethal (5/5 mice) but required ~10 days longer than 10^6 cells to kill the mice.

We have established a suspension-culture line from RFM mice with myeloid leukaemia. The cells are morphologically, histochemically, and karyotypically very similar to our in vivo myeloid leukaemia line. During establishment of the cell line, the leukaemic cells initially required a feeder monolayer for continued replication. Monolayers derived from marrow or spleen or normal mice or mice with mye-
loid leukaemia were equally effective in supporting cell growth. During this period CSF was not detectable in the supernatant. These observations are reminiscent of those of Dexter et al. (1977) who reported maintenance of normal haematopoietic stem cells under similar conditions.

The cell line has a rapid growth rate in vitro, with a high rate of cell death. The cells also grow quite well in diffusion chambers implanted into RFM mice. The cells do not appear to respond to CSF but there does appear to be a low degree of spontaneous maturation along the macrophage pathway.

Whilst the suspension-culture line is morphologically similar to the in vivo line, there are significant differences between the two. Both cell lines have sub-populations of cells with chromosome numbers of 37, 38 and 39. The morphology of the chromosomes of in vivo and in vitro lines differ and will be discussed elsewhere (Kohno et al., in preparation). The most significant difference between the in vitro and in vivo lines relates to the biological difference. The in vivo line is much more malignant than the in vitro line and the latter produces tumours at the site of inoculation (whether i.p. or s.c.) while the former does not. The excellent growth of the in vitro line within diffusion chambers implanted in vivo in RFM mice suggests that these cells may be more antigenic then the parental in vivo line, and thus their growth may be somewhat retarded when placed unprotected (i.e., not within diffusion chambers) in RFM mice.

The establishment of this cell line will simplify studies of RFM myeloid leukaemia by providing a ready supply of leukaemic cells for both in vitro and in vivo studies of the immunology, determinants of drug sensitivity, and relationships between leukaemic and normal haematopoietic stem cells.

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