Effect of the substratum on the growth of CFU-c in continuous marrow culture[1]

M. Tavassoli

Veterans Administration Hospital, University of Mississippi Medical Center, Jackson (Mississippi 39216, USA), October 21, 1982

Summary. The effect of the substratum on the maintenance of granulocyte-macrophage progenitors (CFU-c) was studied in continuous mouse marrow culture. Glass and decalcified eggshell membrane were found preferable to plastic, providing better adherence of stromal cells. Eggshell membrane consists of a meshwork of branching fibers suitable for the adherence of stromal cells. The glass surface with its high surface pH may provide an electrostatic attraction for the negatively charged surface of stromal cells.

Hemopoietic marrow consists of a meshwork of stromal cells upon which hemopoietic stem cells (HSC) differentiate and mature[2]. Exploiting the adherent quality of the stromal cells, an in vitro system has been introduced[3,4] wherein the stromal cells, grown in plastic flasks, are permitted to interact with HSC and to maintain progenitor cells in the supernatant. I now report quantitative data to indicate that 2 other substrata, glass and decalcified eggshell membrane, are preferable to plastic in maintaining progenitor cells in this culture system.

Material and method. Continuous marrow culture was established, as described[3-6], by placing the content of 1 mouse femur (male DBA/2, 6--8-week-old) into plastic flasks or petri dishes containing 10 ml Fischer's medium supplemented with 25% horse serum and 1×10^{-7} M hydrocortisone. Cultures were incubated at 33 °C under 5% CO_2 and populations were reduced by half at weekly intervals. Total cell count and the concentration of granulocyte-macrophage progenitor cells (CFU-c) were studied in the removed supernatant as described[6]. Cultures were studied periodically by phase microscopy and preparations were made for scanning electron microscopy (SEM). After 3 weeks, when a stromal layer had been established, the cultures were seeded by the addition of the content of another femur. All cultures were done in triplicates.

Variable numbers of sterilized round glass coverslips (12 mm) were placed in the culture dishes. Chicken eggshell membrane was decalcified[8,9] in 5% acetic acid, sterilized by autoclaving, cut into round or square pieces and lightly attached to the bottom of culture dishes. Care was taken to prevent overlapping of the coverslips while changing the culture medium. Whereas eggshell membranes adhere well to the bottom of the plastic dish, coverslips are motile particularly during the 1st week. The total surface area of the dish and the area covered by substrates were calculated by tracing the desired area on a filter paper and then cutting and weighing the paper. Three groups of experiments were done in which the 14%, 36% and 52% of the surface area of culture dishes were covered by different substrates.

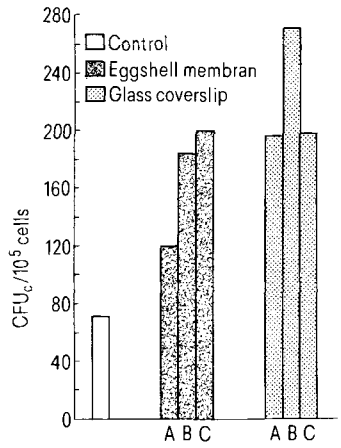

Figure 2. CFU-c concentration in the supernatant at 4 weeks. Control cultures have been compared to these with 14% (A), 36% (B) or 52% (C) surface coverage. Similar pattern was obtained for total cell counts.

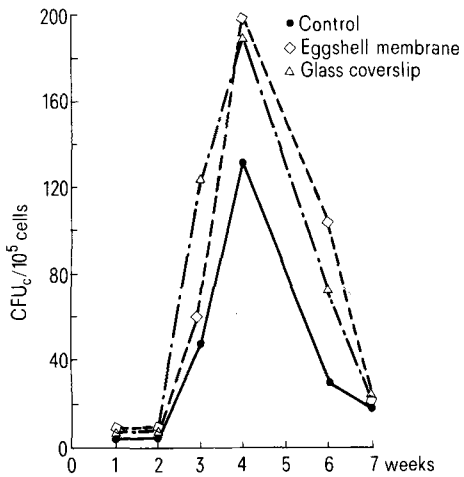

Figure 1. CFU-c concentration in the supernatant as a function of time for control cultures compared to 36% surface coverage with added substrates. Similar patterns were obtained in cultures in which 14% and 52% of surface was covered.

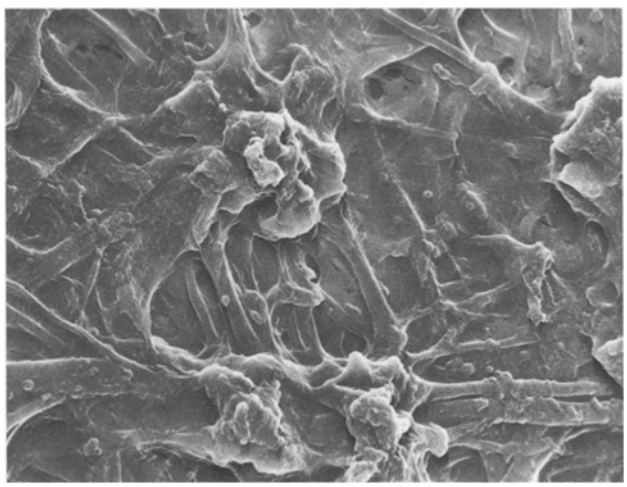

Figure 3. SEM of the eggshell membrane surface. Note the meshwork of branching fibrous structure, variable in thickness and displaying multiple nodes. These can provide a frame for the support of the stromal layer. The flat sheets emanating from the fibrous structures can provide a surface upon which the stromal cells can spread. × 328.

Results and discussion. Both the total cell and CFU-c concentrations increased after seeding the cultures, reaching a peak by week 4 at which time there were consistently higher concentrations in cultures with added substrates. Figure 1 shows CFU-c data for 36% of surface area coverage. The fall in the cell number after week 4 was associated with detachment of the adherent stroma. Figure 2 compares the CFU-c concentration at 4 weeks for all 3 groups. A consistent increase in CFU-c was noted which, in the case of eggshell membrane, appeared to be a function of the proportion of the surface area covered. A similar increase but of higher magnitude was observed with glass coverslips but here the concentration peaked with 36% coverage. The total cell count in the supernatant followed a similar pattern suggesting that the CFU-c concentration was a representative function of HSC in these studies.

Because similar cell numbers were used for the seeding of the cultures, the observed increases can only be attributed to enhanced support potential of the adherent stroma. This can come about because of either qualitative or quantitative difference in the adherent stromal layer or both. A quantitative increase in the density of the adherent stroma was observed on both added substrates using light and SEM. On glass coverslip, stromal cells covered both sides of the substrate in a higher density compared to plastic. The coating of both sides is possible because the coverslip is not tightly attached to the dish. By contrast, the eggshell membrane which was tightly attached to the dish was not coated on both sides, but the high cell density was evident. Structurally the glass surface appeared smooth on SEM, but the eggshell membrane appeared as a meshwork of branching fibrous structures, variable in thickness displaying multiple nodes from which emanated flat sheets which merged with the fibrous structures (fig. 3). This may provide a physical frame to support the adherence of stromal cells.

In glass surface, the higher density of adherent cells may simply be a pH effect[9]. Glass provides a relatively high pH of about 9. The cell surface, on the other hand, is negatively charged as demonstrated by strong staining with the positively-charged polycationic ferritin[4]. An electrostatic attraction may thus give a higher density of adherent cells on the glass, compared to plastic.

1 Supported by NIH grant No. 30142 to M. T.
2 Tavassoli, M., Exp. Hemat. *3* (1975) 213.
3 Dexter T.M., Allen, T.D., and Lajtha, L.G., J. Cell Physiol. *91* (1977) 335.
4 Dexter, T.M., and Testa, N.G., Meth. Cell Biol. *14* (1976) 387.
5 Tavassoli, M., and Takahashi, K., Am. J. Anat. *164* (1982) 91.
6 Tavassoli, M., Exp. Hemat. *10* (1982) 435.
7 Leighton, J., Mansukhani, S., and Estes, L.W., In Vitro *6* (1971) 251.
8 Leighton, J., Abaza, N., and Mansukhani, S., in: Tissue culture, methods and applications, p. 452. Eds P.F. Kruse and M.K. Patterson. Academic Press, New York 1973.
9 Bessis, M., Living blood cells and their ultrastructure, p. 146. Springer, Berlin 1973.

0014-4754/83/040411-02$1.50 + 0.20/0

Heat(40 °C)-induced polypeptides in human embryonic fibroblasts

Y. Nishiyama, Y. Tsutsui, T. Tsurumi, H. Aoki, Y. Ito and K. Maeno

Department of Microbiology, Germfree Life Research Institute, and Department of Pathology, Nagoya (Japan), and University School of Medicine, Nagoya (Japan), April 15, 1982

Summary. The synthesis of 3 polypeptides with molecular weights of 103-, 80- and 78-kdalton (K) was dramatically accelerated when the cultures of human embryonic fibroblasts were transferred from 37 to 40 °C. The induction of these polypeptides was not observed if actinomycin D was added to the cultures at the beginning of the rising of the temperature, indicating that this response may be mediated by increased transcription of their messenger RNA.

Recent studies have shown that a number of organisms induce the synthesis of a new set of polypeptides in response to elevated temperature[1-5]. The most extensively studied case is the heat-shock response of *Drosophila melanogaster* cells, and an analysis of the mechanisms has demonstrated that the heat-induced changes in protein synthesis may be mediated by a control acting at the level of translation as well as transcription[3]. We present here clear evidence to indicate that human cells are also capable of inducing preferential synthesis of a small number of polypeptides in response to elevated temperature.

Materials and methods. Human embryonic fibroblasts (HEF) were prepared from 3- to 5-month-old fetuses and grown at 37 °C in Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum, 100 units/ml of penicillin and 100 µg/ml of streptomycin. Two human tumor cell lines, Hela and Calu-1 cells, were also used in this study. The latter cell line of lung origin was kindly supplied by Dr Jørgen Fogh, Sloan-Kettering Institute for Cancer Research, New York. Polyacrylamide slab-gel electrophoresis (PAGE) was carried out by the method of Laemmli[6].

Samples were dissociated in 0.0625 M Tris-HCl (pH 6.8) containing 5% SDS, 2% 2-mercaptoethanol, 10% glycerol, and 0.001% bromophenol blue, followed by heating at 100 °C for 1 min. RNA polymerase (165-K, 155-K, 39-K), bovine serum albumin (68-K) and soybean trypsin inhibitor (21-K) were used as reference protein markers. After electrophoresis, the gels were fixed, dried and then exposed to Kodak Royal X-Omat films at −80 °C. Scanning of the film was carried out by a Shimazu dual-wavelength TLC scanner CS-910.

Results and discussion. As shown in figure 1, the incubation of HEF at 40 °C resulted in a change in the pattern of protein synthesis; the synthesis of polypeptides of sizes larger than 110-K was generally reduced whereas the synthesis of most polypeptides of sizes smaller than 80-K was slightly enhanced. The most striking change, however, was a dramatic increase in the synthesis of 3 polypeptides with molecular weights of 103-, 80- and 78-K. Essentially