Derivation of a New B-Cell Line from Mouse Spleen and Its Characterization

M. V. Raevskaya

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 119, № 2, pp. 183-186, February, 1995
Original article submitted March 15, 1994

Prolonged culturing of splenocytes of (CBA×C57Bl/6)F_1 hybrid mice in RPMI-1640 medium with 15% fetal calf serum resulted in the derivation of a new B-cell line. After a monolayer of stromal fibroblast-like cells was formed, lymphoid cell growth was observed in 4-5 weeks of splenic cell culturing. All the cells of the splenic line belonged to the B series and expressed IgM on their surface; they did not form lymphoid colonies after injection to lethally irradiated mice, and even after 8-month culturing and several passages they could not be differentiated.

Key Words: *B-cell line; spleen; lymphopoiesis*

Culturing of mouse splenic cells in RPMI-1640 medium resulted in the derivation of a lymphoid cell line capable of prolonged self-maintenance. The purpose of this research was to characterize the resultant cells and select the conditions for their prolonged culturing *in vitro* and *in vivo*. Culturing of mouse bone marrow cells in RPMI-1640 medium has been shown to lead to prolonged normal B lymphopoiesis [7-9]. Attempts to achieve B lymphopoiesis by splenocyte culturing have failed. Our task was to elucidate the type of lymphopoiesis in splenocyte culture: whether it is normal B lymphopoiesis or a transformed strain of lymphoid cells.

MATERIALS AND METHODS

(CBA×C57Bl/6)F_1 hybrid mice aged 2 to 3 months bred at the Stolbovaya breeding center were used in the experiments.

For the preparation of a B-cell line from two mouse spleens isolated under aseptic conditions, a cell suspension was prepared, filtered through 4 capron layers, and poured into Nunc culturing flasks, 2×10^6 cells/ml. The cells were cultured in RPMI-1640 medium (Flow Lab.) with 15% fetal calf serum, 200 mM L-glutamine (Serva), 2×10^{-5} M 2-mercaptoethanol, and gentamicin (50 mg/ml). The medium was replaced every 3-4 days.

Bone marrow and splenic stromal cell cultures grown routinely were used as the basal layer [3-5]. The concentration of cells for culturing was 1×10^6 cells/ml. RPMI-1640 medium for culturing contained 10% fetal calf serum and 200 mM L-glutamine. After the appearance of fibroblast-like cell colonies (after 7-14 days of culturing) the culture medium was replaced with fresh medium and 2×10^6 cells grown from the spleen were added to each flask.

To animals treated with pristan in a dose of 0.2 ml, 5×10^6 cells of a splenic line (SL) were injected intraperitoneally.

Twenty-five mice were irradiated at 950 rad using an EKU-50 device (dose rate 85 rad/sec). Twenty-four hours later the animals were intravenously injected SL cells in a dose of 10^6, 3×10^6, and 5×10^6. After 9 days the animals were narcotized and histologic preparations were made from the spleens by the standard method [6].

SL cell smears were prepared and stained by the standard method with methanol fixation and staining with May-Grunwald and Giemsa solutions.

Department of Cell Physiology and Immunology, Biological Faculty, M. V. Lomonosov State University, Moscow. (Presented by I. P. Ashmarin, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0002-0176$12.50

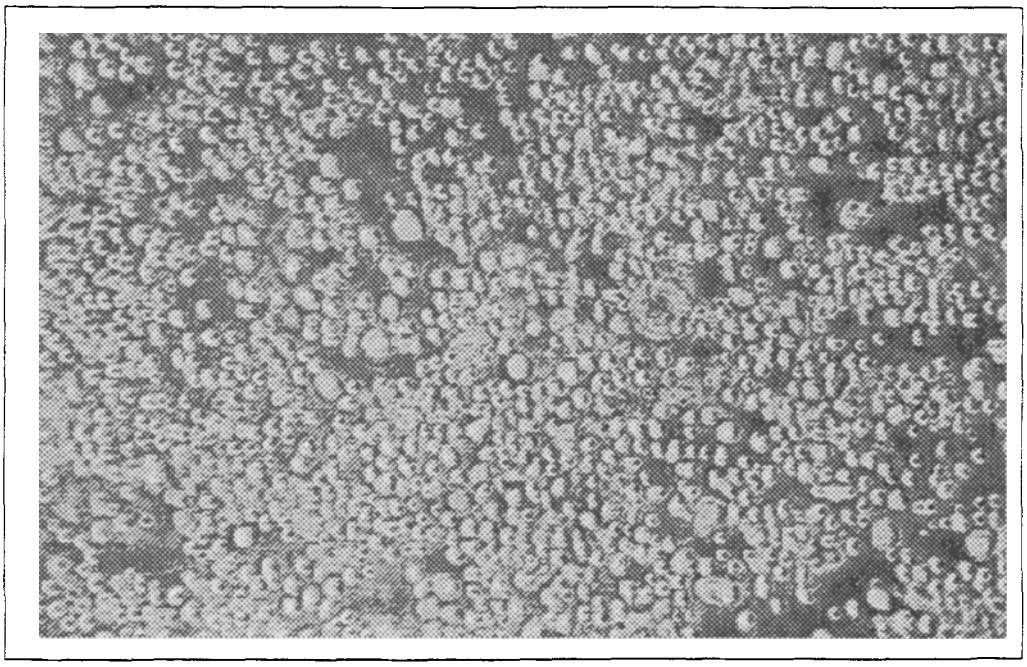

Fig. 1. Live SL cell culture 7 days after passage onto the underlayer. Underlayer: 7-day primary culture of splenocytes. ×20.

The cytotoxic test for assessment of Thy-1$^+$ SL cells was performed as described previously [1].

Direct immunofluorescence for estimation of Ig$^+$ SL cells was carried out with commercial fluorescent rabbit antiserum to murine immunoglobulins (Ig) [1].

The cell membrane Ig isotype was assessed by enzyme immunoassay. For this purpose, 50 μl of 0.1% poly-L-lysine was added to Nunc polystyrene plates for microtitration and incubated 1 h at 37°C. Cells were then added: 1.5×10^4 cells/well in 50 μl phosphate buffer saline. The cells were sedimented by 5-min centrifugation at 250 g, and 50 μl 1% glutaraldehyde were added to attain 0.5% concentration. Enzyme immunoassay was then carried out routinely [2] with rabbit monospecific antisera to mouse IgA, IgG1, IgG2a, IgG2b, IgG3, and IgM (Miles). The substrate of the enzymatic reaction was *o*-phenylenediamine. The results were scintillated using Multiscan-Elisa at wavelength 492 nm.

RESULTS

The growth of small fibroblast colonies in the culture, hemopoietic and lymphoid cells was observed 5-7 days after explantation of the suspension of intact splenocytes of $(CBA\times C57Bl/6)F_1$ hybrid mice. By day 20 of culturing the hemopoietic and lymphoid cells were virtually destroyed and the bottom of the flask was covered with a layer of fibroblast-like cells. After 30 days a small focus of round cells appeared in one of the flasks on a monolayer of fibroblast-like cells, which clearly differed in morphology from the underlying elements. The number of cells in this focus increased with culturing. After one week, foci of round cells occupied one-third of the flask bottom, and eventually these cells covered the bottom completely. Numerous floating cells were observed (Figs. 1, 2). The size and shape of adhered and floating cells varied: from foci with just a few round cells to large foci with round and elongated

TABLE 1. SL Cell Culturing in Different Culture Media on Different Underlayers

Culture medium	Underlayer, fibroblasts	SL cell growth
RPMI-1640	–	Adhere to plastic, grow intensively
RPMI-1640	Splenic and thymic	Adhere to the underlayer, grow intensively
RPMI-1640	Bone marrow	Do not adhere to the underlayer, destroyed in 7 days
α-MEM	–	Destroyed in 5-7 days
α-MEM	Splenic	Detached from the underlayer, destroyed in 5-7 days
α-MEM	Bone marrow	Do not adhere to the underlayer, destroyed in 5-7 days
Iscove's	–	Destroyed in 3 days
Iscove's	Splenic	As above

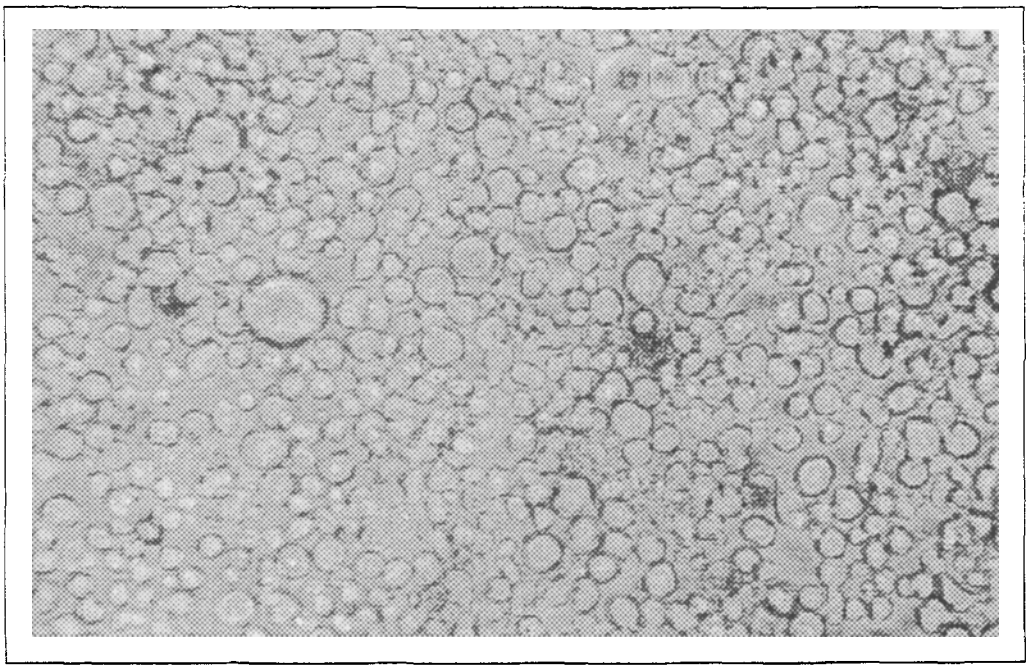

Fig. 2. The same SL culture. ×70.

cells of different size. Morphologically, two types of cells were distinguished: with clear-cut and poorly discernible basophilia of the cytoplasm. All the cells contained a large nucleus and cytoplasm with numerous processes of the cell membrane. Floating cells were transplantable to new culture flasks with and without an underlayer of splenic stromal cells. Transfer of SL cells to α-MEM culture medium led to the death of cells with their fragmentation and ejection of the nuclei. Attempts to maintain cells in Dulbecco's medium modified by Iscove also failed.

SL cells cultured on an underlayer of bone marrow stromal cells did not adhere to the underlayer and died in the course of culturing (Table 1).

Transplantation of SL cells to pristane-treated mice did not result in tumor formation.

Transplantation of SL cells to mice preirradiated with 950 rad did not lead to the formation of colonies of hemopoietic and lymphoid cells in the spleen, although the viability of irradiated animals increased.

The cytotoxic test did not detect Thy-1$^+$ cells. The immunofluorescent test showed 100% fluorescence of SL cells. According to the type of fluorescence, 90% were cells with a uniform surface fluorescence. Moreover, besides the surface, intracellular granules fluoresced in some cells. After 1-2 months of culturing and passages only cells with uniform surface fluorescence were seen. All SL cells contained surface IgM even after 6 to 8 months of culturing and passages.

Hence, we derived a new, self-sustaining line of lymphoid cells cultured in RPMI-1640 medium which is not maintained in Iscove's and α-MEM media. All the cells of this line fluoresce in the immunofluorescent test with rabbit antiserum to mouse immunoglobulins and carry IgM on their surface, this permitting us to classify the new line as B-cellular. Despite the failure of attempts at inducing tumor formation by intraperitoneal injection of the cells, it seems that we can speak of a transformed B-cell line. Prolonged (12 months and more) transplantability of cells without substrate and the absence of differentiation in the course of culturing provide evidence in favor of this assumption. With normal B lymphopoiesis, cell differentiation is observed after 3 to 5 months of culturing [7-9].

The author is grateful for the assistance provided by N. I. Sharova, I. A. Miroshnichenko, and I. A. Nikolaeva from the Institute of Immunology, Russian Ministry of Health.

REFERENCES

1. G. Frimel' (Ed.), *Immunological Methods* [in Russian], Moscow (1987).
2. *Immunological Methods,* eds. I. Lefkovits and B. Pernis, Academic Press (1979).
3. A. J. Friedenstein, R. K. Chailakhyan, N. V. Latsinic, *et al., Transplantation,* **17**, 331-340 (1974).
4. A. J. Friedenstein, U. F. Gorscaja, and N. N. Kulagina, *Exp. Hemat.,* **4**, 267-274 (1976).
5. A. J. Friedenstein, *Bone Mineral Res.,* 7, 243-272 (1990).
6. J. E. Till and E. A. McCulloch, *Proc. Nat. Acad. Sci. USA,* **51**, 29 (1964).
7. C. A. Whitlock and O. N. Witte, *Ibid.,* **79**, 3608 (1982).
8. C. A. Whitlock, S. F. Ziegler, L. J. Treiman, *et al., Cell,* **32**, 903 (1983).
9. C. A. Whitlock, D. Robertson, and O. N. Witte, *J. Immunol. Meth.,* **67**, 353 (1984).