

LONG-TERM ESTABLISHMENT OF A HUMAN PLASMACYTE CELL
LINE DERIVED FROM A PATIENT WITH
IgD MULTIPLE MYELOMA

I. REQUIREMENT OF A PLASMACYTE-STIMULATING FACTOR FOR
THE PROLIFERATION OF MYELOMA CELLS IN TISSUE CULTURE*

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The establishment of cell lines from mouse and human myelomas has been attempted by a number of laboratories (1-6). Cultures of mouse myelomas have been successfully established with the use of feeder cells (2, 3) and with the use of a phagocytic cell factor (1). Feeder cells and proliferation factors were found to be essential components for the proliferation of antibody-forming cells.

Furthermore, the role of cell-to-cell contact has been implicated in culture initiation and colony formation of lymphocytes (7-9). In spite of these results, the establishment of a long term culture of human multiple myeloma plasmacytes has been relatively unsuccessful.

Both the lymphocyte and the plasmacyte are vitally involved in the immune response. Even though biochemically the proteins produced by both cell types are identical, there are several unique differences between the morphological patterns of lymphocytes (10, 11) and plasmacytes (12, 13).

We felt it important to establish a human plasmacyte cell line to compare to normal lymphoid cell lines established in our laboratory. This report describes the methods of cultivation, morphology, and proliferative characteristics of a plasmacyte cell line derived from the tumor cells of a patient with IgD lambda multiple myeloma.

Materials and Methods

Myeloma cells were obtained by removal of fluid from the right post thorax of a 59-yr old female with a 3 yr history of IgD myeloma. A light chain of the lambda type was found in the serum, and a large amount of lambda-type Bence-Jones protein was detected in the urine. Skeletal lesions characteristic of multiple myeloma were also present.

Initiation and Maintenance of Cultures.—Pleural fluid (1,500 ml) was collected in a sterile

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flask containing 10,000 U of sodium heparin in 50 ml of Earle's balanced salt solution (EBSS)¹ without Ca⁺⁺ or Mg⁺⁺. The heparinized pleural fluid, containing 1,400 white blood cells (WBC)/m³, was divided into 250-ml samples and centrifuged at 1,500 rpm for 10 min. The fluid was removed from the tubes by aspiration, and the cell pellets were washed twice with EBSS (Ca⁺⁺ and Mg⁺⁺ free).

The pellets were resuspended in various media to a concentration of 7×10^6 /ml, and half of each cell suspension was inoculated into 250-ml Falcon flasks (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). The remaining half of each suspension was inoculated into similar flasks containing an agar substrate overlay described below. All of the flasks were incubated in an atmosphere of 5% CO₂ and 95% air at 37°C.

The cultures were fed twice weekly by standing the flasks on end in the incubator to allow the cells to settle. One-half of the medium from each flask was removed by aspiration and replaced with fresh medium to the original volume.

Preparation of Agar Substrate Overlay.—The bottom of each flask was covered with 7 ml of an agar solution containing one part 3% agarose in three times distilled pyrogen-free water (sterilized by autoclaving); one part EBSS without Ca⁺⁺ or Mg⁺⁺; and one part fetal calf serum (FCS) which was heat inactivated at 56°C for 30 min. The agar was dissolved by boiling and allowed to cool in a 56°C water bath before the other ingredients were added. The liquid agar solution was spread over the bottom of each flask and the flasks were left at room temperature until the agar solidified. The flasks were stored upside down at 4°C. The media and cells were added to the flask directly onto the agar surface.

Nutritional Media.—RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.), Eagle's MEM, and Dulbecco's modified media were used in separate attempts to initiate cell growth. These media were supplemented with either FCS or human AB (HAB) serum (heat inactivated for 30 min. at 56°C), 1% glutamine, and 1% fungizone and penstrep (GIBCO). Cultures were kept at a pH of 6.6–6.8 by adjusting with sterile HCl or NaOH.

Cell viability remained low after 1 mo of cultivation. Therefore the cells were spun down and resuspended in special RPMI 1640 base medium MJLA-1 (Table I) and supplemented with the following: 50 µg of gentamicin reagent (Microbiological Associates, Inc., Los Angeles, Calif.), 1% fungizone, and 40% FCS (Reheis Chemical Co., Chicago, Ill.).² Cells were resuspended in the new medium to half the original volume and returned to the same flasks.

Preparation of Fibroblast "Feeder Cells" and Fibroblast Conditional Medium (FCM).—Fibroblasts that grew out in the original culture were replaced by cultured irradiated human normal skin fibroblasts. Normal fibroblasts obtained from two donors were separately cultured until monolayers formed. Each culture was trypsinized and resuspended in medium to a concentration of 10⁶/ml. 7 ml from each cell suspension were added to separate 250-ml flasks (14, 15) before irradiation with 2,000 rads of cobalt-60. The irradiated cells were incubated overnight at 37°C in an atmosphere of 5% CO₂ and 95% air. Myeloma cells were added to the flasks after incubation.

FCM was obtained from a monolayer culture of normal human skin fibroblasts. FCM was removed from the culture cells on the 3rd day after feeding. The medium was spun for 10 min at 1,500 rpm and filtered through a 0.45 µm Nalgene filter (Nalge Co., Nalgene Labware Div., Rochester, N. Y.).

The activity of the stimulating factor contained in the FCM was determined by serial dilutions of the media. 1 ml of the dilutions was added to 1 ml of the MJLA-1 medium contain-

¹ *Abbreviations used in this paper:* EBSS, Earle's balanced salt solution; FCM, fibroblast conditional medium; FCS, fetal calf serum; HAB, human AB serum; PSF, plasmacyte-stimulating factor; WBC, white blood cells.

² 12 lots of FCS from various companies were tested on these cells, and Reheis was found to be least toxic.

TABLE I
Media MJLA-1

Media ingredients	mg/liter	Media ingredients	mg/liter
L-arginine	305.0	Biotin	0.20
L-cystine (di HCl)	81.0	Vitamin B ₁₂	0.005
L-histidine	46.0	Calcium pantothenate	0.25
L-isoleucine	70.5	Choline chloride	3.0
L-leucine	102.5	Folic acid	1.0
L-lysine	98.0	<i>l</i> -inosital	35.0
L-methionine	30.0	Nicotinamide	1.0
L-phenylalanine	47.0	Para amino benzoic acid	1.0
L-threonine	68.0	Pyridoxine HCl	1.0
L-tryptophane	15.0	Rivoflavin	0.20
L-tyrosine (Na)	25.7	Thiamine HCl	1.0
L-valine	66.0	Glutathionine reduced	1.0
L-glutamine	884.0	Dextrose	2,000.0
L-alanine	1.78	NaCl	6,000.0
L-aspartic acid	46.6	Potassium Cl	400.0
L-asparagine	80.0	NaHCO ₃	2,200.0
L-glutamic acid	49.4	Na ₂ HPO ₄	1,512.0
L-proline	43.0	MgSO ₄ ·7H ₂ O	100.0
L-serine	51.0	Ca(NO ₃) ₂ ·4H ₂ O	100.0
Glycine	25.0	Phenol red	5.0

ng 1×10^5 viable myeloma cells. The cells were incubated at 37°C (5% CO₂ 95% air) in 15-ml culture tubes standing upright. The viabilities and cell counts were read daily for 10 days on a cytograf (Bio/Physics Systems Inc., Mahopac, N. Y.) in the presence of trypan blue. The titer of the stimulating factor was expressed as the highest dilution which yields maximal growth over a 10-day period. During this period the cells were fed twice weekly by removing half the volume and replacing it with fresh media.

Conditioned media of different kinds were also obtained from five established lymphoid cell lines, two macrophage cell lines, and one thymus epithelial cell line, all of which were derived from normal human tissue. The growth-stimulating activity of each of these media was assayed by adding 1 ml of conditioned media to 1 ml of myeloma cell suspension containing 1.5×10^5 viable cells/ml. These cells were incubated as above for 3 days. The viability and cell counts were recorded as before.

Isolation and Identification of Plasmacyte-Stimulating Factor (PSF).—Isolation of PSF was carried out by first concentrating FCM ten times with a Diaflow Ultrafilter (Amicon Corp., Lexington, Mass.) containing a PM10 membrane. The concentrated medium was fractionated on Sephadex G-100 and G-200 columns (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) equilibrated with 1.5% Tris-NaCl buffer at pH 8.6. The columns were calibrated with ovalbumin, bovine albumin, and aldolase before the media was fractionated. Conditioned media from lymphoid cell lines and fresh media were used as controls. After the optical density of each fraction was determined, the fractions were sterilized by filtering through a .22 micron millipore filter. The sterile fractions were added to 1×10^5 viable myeloma plasmacytes containing 1 ml of MJLA-1 media (pH 7) (Table I). The cells were incubated for 48 h at 37°C in 5% CO₂ and 95% air. Cell counts and viability were recorded every 12 hr. The population distribution, cell size changes, cell counts, and viability of this assay were measured on a cytograf.

Heat inactivation was carried out on Fraction no. 20 of the FCM collected from the Sephadex G-200 column. The fraction was divided into six portions and heat inactivated for 30 min at the following temperatures: 30°, 40°, 50°, 60°, 70°, and 80°C. Trypsin digestion was performed on Fraction no. 20 containing 200 µg/ml trypsin and incubated for 3 h at 37°C. The reaction was stopped by adding 500 µg/ml soybean trypsin inhibitor. The heat-inactivated and trypsin-digested fractions were sterilized as above and assayed back on 1×10^6 plasmacytes/ml. Cell counts and viability were performed after 72 hours and the percent increase over the original cell count was recorded.

Chromosome Studies.—The effects of fibroblasts or FCM on myeloma plasmacytes were studied on cells that were grown fibroblast-free or FCM-free for 2 wk. The mitotic coefficient was calculated before and 24 h after the myeloma cells were added to new fibroblasts. The cells were then fixed with 1:3 acetic acid and alcohol, and flame dried to spread the chromosomes (16, 17). The chromosome spreads were stained for 5 min in 10% Giemsa stain and 90% buffered H₂O. The slides were surveyed under oil emersion; 1,000 cells were counted on each slide and the number of cells in metaphase recorded. The cells in metaphase were also examined for abnormal karyotypes.

Identification of Immunoglobulin Synthesized by the Myeloma Cells in Tissue Culture.—The culture fluid was collected and analyzed with immunoelectrophoresis and the Ouchterlony method, and compared with the patient's serum and pleural fluid using specific antisera against IgG, IgA, IgM, and IgD and kappa and lambda chains (Meloy Laboratories Inc., Springfield, Va.). Further analysis of the cultured cells was made by [³H]leucine incorporation studies.

Electron Microscopy.—Cells for transmission electron microscopy were pelleted by centrifugation at 1,500 rpm for 10 min and fixed in 3% glutaraldehyde in cacodylate buffer. The pellets were dehydrated in ethanol and embedded in epoxy (Spurr), then osmicated and post fixed with urinal acetate and lead.

Cells for scanning electron microscopy were cultured on 25-mm diameter cover glasses placed in 35 × 10 Falcon petri dishes. Irradiated fibroblasts were added to the petri dishes and allowed to attach to the cover glasses. Myeloma cells were then added and the cultures were incubated for 2 days. The cells on the cover glasses were fixed in glutaraldehyde, osmicated, and dehydrated with ethanol in situ. A cover glass bearing the ethanol-saturated cells was inserted in a special cover glass holder and dried in CO₂ by the critical point method (18). The dry monolayer was coated with successive layers of carbon and gold.

RESULTS

Initiation of a Continuous Culture.—The myeloma plasmacytes were cultured for 1 mo in Eagle's MEM, RPMI 1640, and Dulbecco's medium (Table II). As can be concluded from Table II, establishment of a continuous culture of myeloma plasmacytes was possible only in the presence of fibroblasts overlaid with agar. The myeloma cells formed colonies in the agar over the fibroblasts approximately 1 mo after the initiation of the cultures. By the 2nd mo of cultivation, the agar had been digested and the myeloma cells had formed aggregates attached to the fibroblasts.

The media used initially (Table II) supplemented with heat-inactivated FCS, however, were not ideal. Viability and cell counts remained low. The addition of large amounts of amino acids (media MJLA-1, Table I) increased both viability and cell count. Under these conditions, viable cell counts eventually increased to 80–90% at 3.5×10^6 .

By the 4th mo, the fibroblasts began to decrease in viability and cell count.

TABLE II
Initiation of Plasmacyte Cell Line LA no. 49

Initiation media	% Viable 1 mo late	% Viable 16 days after addition of MJLA-1
1. RPMI 1640 + FCS ₄₀	0	0
2. RPMI 1640 + FCS ₄₀ + agar	15	58
3. RPMI 1640 + HAB ₄₀	3	2
4. RPMI 1640 + HAB ₄₀ + agar	12	50
5. Dulbeccos + FCS ₄₀	0	0
6. Dulbeccos + FCS ₄₀ + agar	10	50
7. Dulbeccos + HAB ₄₀	0	0
8. Dulbeccos + HAB ₄₀ + agar	7	12
9. MEM + FCS ₂₀	0	0
10. MEM + FCS ₂₀ + agar	0	0

The above media were supplemented with either 40% FCS or 40% HAB sera. Fibroblasts grew out in culture numbers 2, 4, 6, and 8. MEM supplemented with FCS and agar overlay was still not rich enough in amino acids to support growth of the plasmacytes.

The myeloma plasmacytes were removed from the nonviable fibroblast culture and added to freshly irradiated fibroblasts. Since the fibroblasts were irradiated in order to control overgrowth, the concentration of FCS was dropped to 20%. Cell count and viability again increased in the presence of irradiated fibroblasts. The mitotic coefficient of the plasmacytes was less than 1% after the death of the fibroblasts. After transferral of plasmacytes to new fibroblasts they began to proliferate almost synchronously, reaching a mitotic coefficient of 36.4% in metaphase by 24 h. The myeloma plasmacytes continued to proliferate for 2 mo until the death of the fibroblasts.

Immunoglobulin Characterization.—The pattern of immunoglobulin synthesis by the cells maintained in culture was the same as that from the original tumor as shown by immunoelectrophoresis and Ouchterlony comparison (Fig. 1) with specific antisera. Biosynthetic study of the established cell line LA-49 with [¹⁴C]leucine demonstrated synthesis and secretion of IgD myeloma protein. The myeloma plasmacytes still secreted the myeloma protein IgD lambda 1 yr after cultivation. The media, concentrated three times, produced a visible line of precipitation when tested against specific anti-IgD sera by the Ouchterlony technique.

Light and Electron Microscopy of Cultured Myeloma Cells—Examination of myeloma plasmacytes from patient and culture by light microscopy revealed no morphological differences between cells from the two sources (Fig. 2). Both original and cultured myeloma cells were similar in size, ranging from 15 to 20 μ m. They were multinucleated and polyploid, with chromosome counts varying from 23 to over 250 (Fig. 3). Most of the cells contained one or more large hyaline bodies. Plasma cells in all stages of maturation, from immature blasts to mature plasmacytes, were found in the cultured cells.

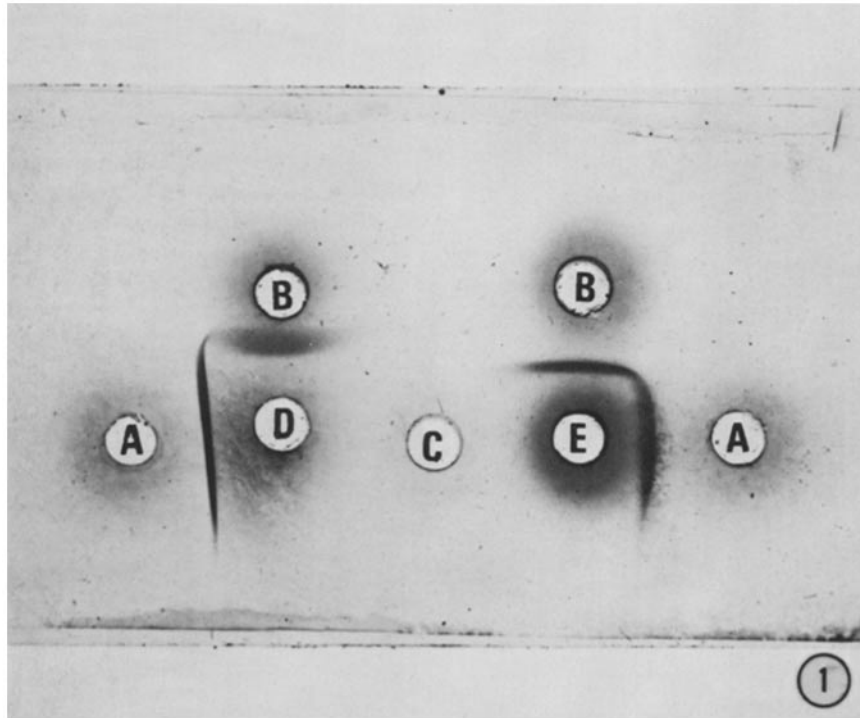


FIG. 1. Demonstration of D lambda myeloma protein from LA-49 culture fluid. A, SE pleural fluid (with D myeloma protein); B, LA-49 culture fluid (at 9 mo); C, LA-85 culture fluid (IgG- and IgM-producing line); D, specific antisera for IgD; and E, specific antisera for lambda light chains.

The cultured myeloma cells were obviously of plasma cell origin. They were easily distinguished from other cells of the lymphoid series by their large round nucleus and basophilic cytoplasm containing a well developed ergastoplasm (Figs. 2 and 4).

Scanning electron microscopy of myeloma cells cultured with fibroblasts reveals a spherical rough-surfaced cell attached to the smooth-surfaced fibroblast (Fig. 5). The fibroblast played host to one or more plasmacytes. The irregularities on the surface of the plasmacytes probably represent protrusions of vesicles, convolutions of the cell membrane, and microvilli. Cell membrane irregularities of this kind can be seen in thin sections of myeloma cells (Fig. 4).

The mechanism by which the myeloma cells are attached to the fibroblast is unknown. The attachment is not secure because clumps of plasmacytes often break free of the fibroblast and resuspend in the medium. Entanglement of surface irregularities of the myeloma cells with microvilli of the fibroblast may be the mechanism of attachment.

The micro morphology of the cultured plasmacytes as seen in thin sections

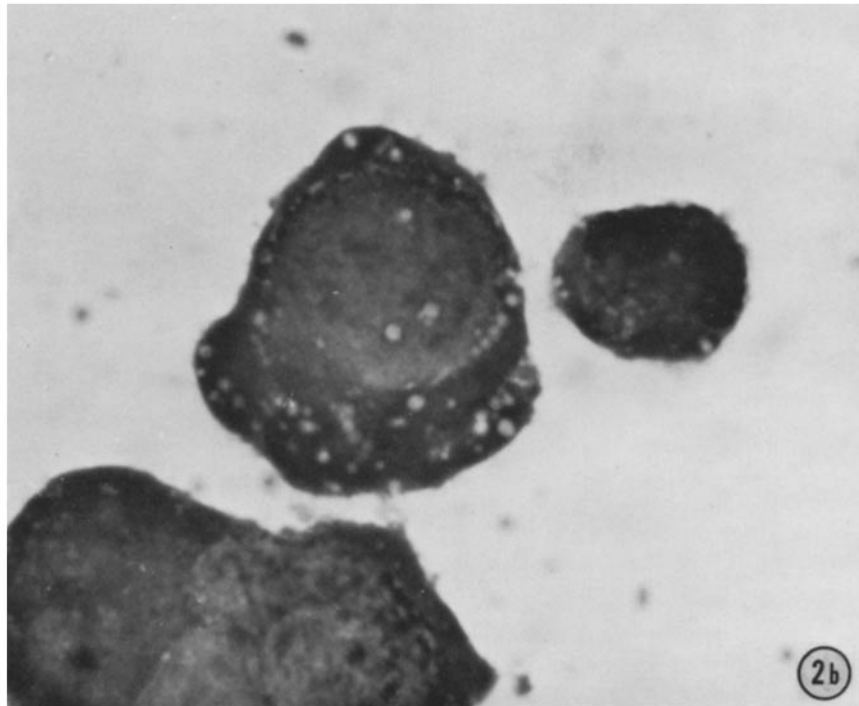
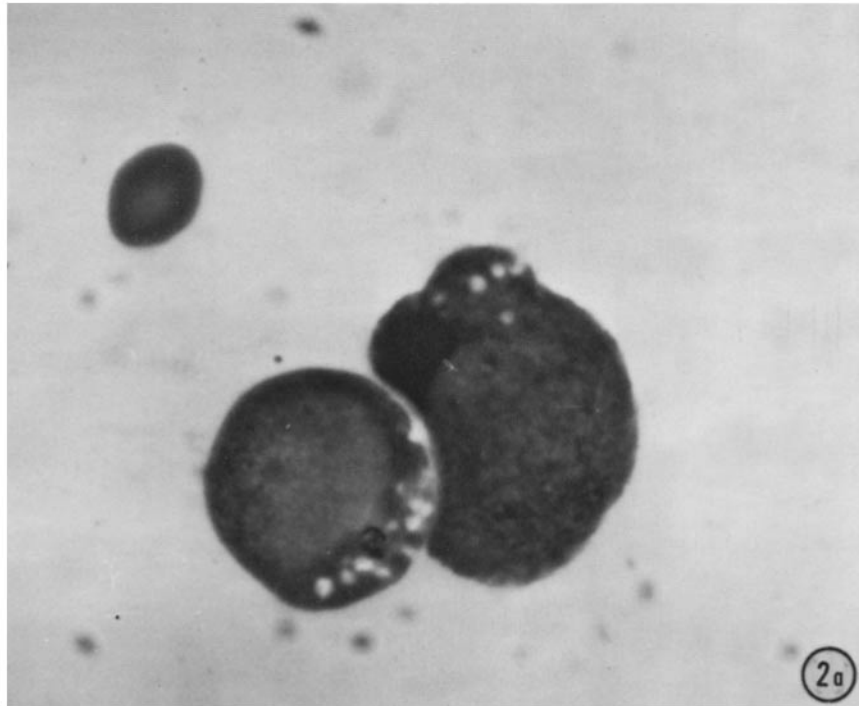


FIG. 2. (A) Plasmablasts containing cytoplasmic vacuoles (Mott cells) isolated from the pleural fluid upon initiation of culture. (B) Plasmablasts 9 mo after initiation of culture. Intranuclear inclusions, multinucleation, and Mott vaculation were characteristic of these cells throughout the course of cultivation.

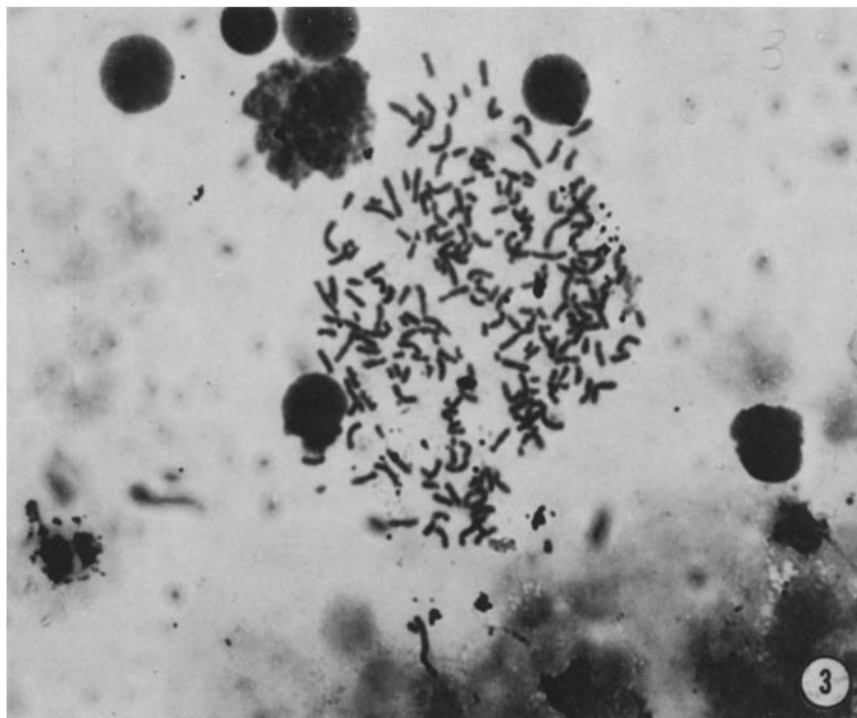


FIG. 3. Metaphase chromosome pattern of cultured cells. This plate contained extremely high number of chromosomes, approximately 250.

was typically that of the plasma cell: an eccentric nucleus with clumped granular chromatin and cytoplasm filled with parallel arrays of granular endoplasmic reticulum. Most of the cultured myeloma cells contained many large mitochondria and a few of the cells contained numbers of lipid bodies (Fig. 4), a not unusual finding for cultured cells.

Substitution of Fibroblasts with FCM Containing a Stimulating Factor.—It was observed that the media taken from a normal fibroblast cell line could also stimulate the myeloma plasmacytes. Fig. 6 represents growth curves of the plasmacytes with irradiated fibroblasts, or FCM, as compared to controls containing only media. In the presence of fibroblasts, the myeloma plasmacytes reached a maximal cell count of 3×10^6 . In the presence of FCM the myeloma cells reached a maximum of 6×10^5 viable cell count, while the cell controls without either eventually had total cell death. Attempts to concentrate the cells in the controls in order to reach a higher viable count still failed to induce proliferation of the culture.

The mitotic coefficient 24 h after the addition of FCM was the same as that of the cultures containing fibroblasts. The highest dilution of the FCM that

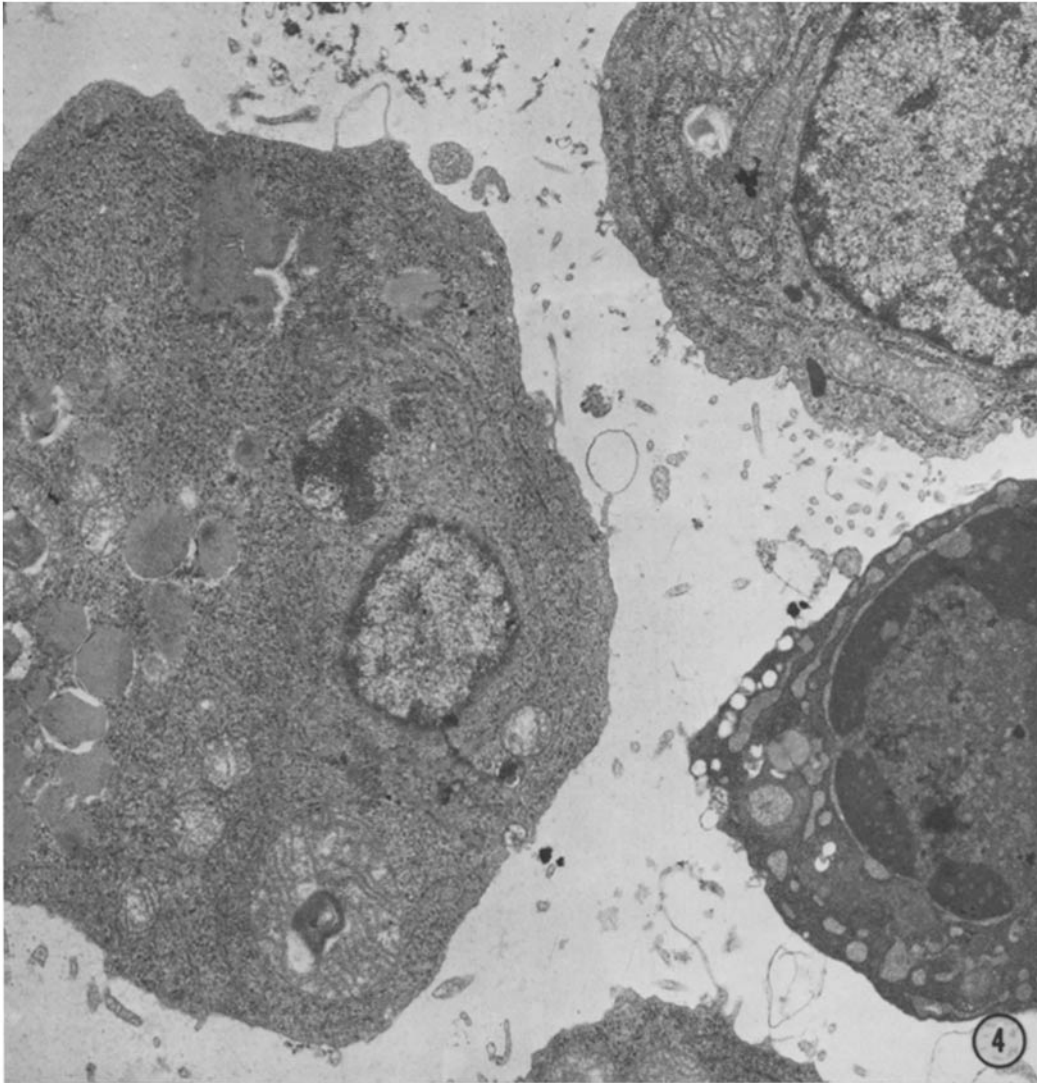


FIG. 4. A section of cultured plasmacytes in various stages of maturation. A blast stage is at the lower right, a mature plasmacyte is at top right, and an older degenerating plasma-cyte is at the left. The eccentric nucleus with marginated clumps of chromatin and parallel rough endoplasmic reticulum are characteristic of the plasma cell. Numbers of lipid bodies and myelinated mitochondrial membranes in the cell on the left are suggestive of degenerative processes. $\times 12,000$.

could stimulate cell growth was found to be 1:1024. The greatest stimulation was produced when the factor was diluted from 1:2 to 1:16.

Conditioned media from the other cell types had no effect upon the growth or the mitotic coefficient of the plasmacytes. In fact, almost total cell death of

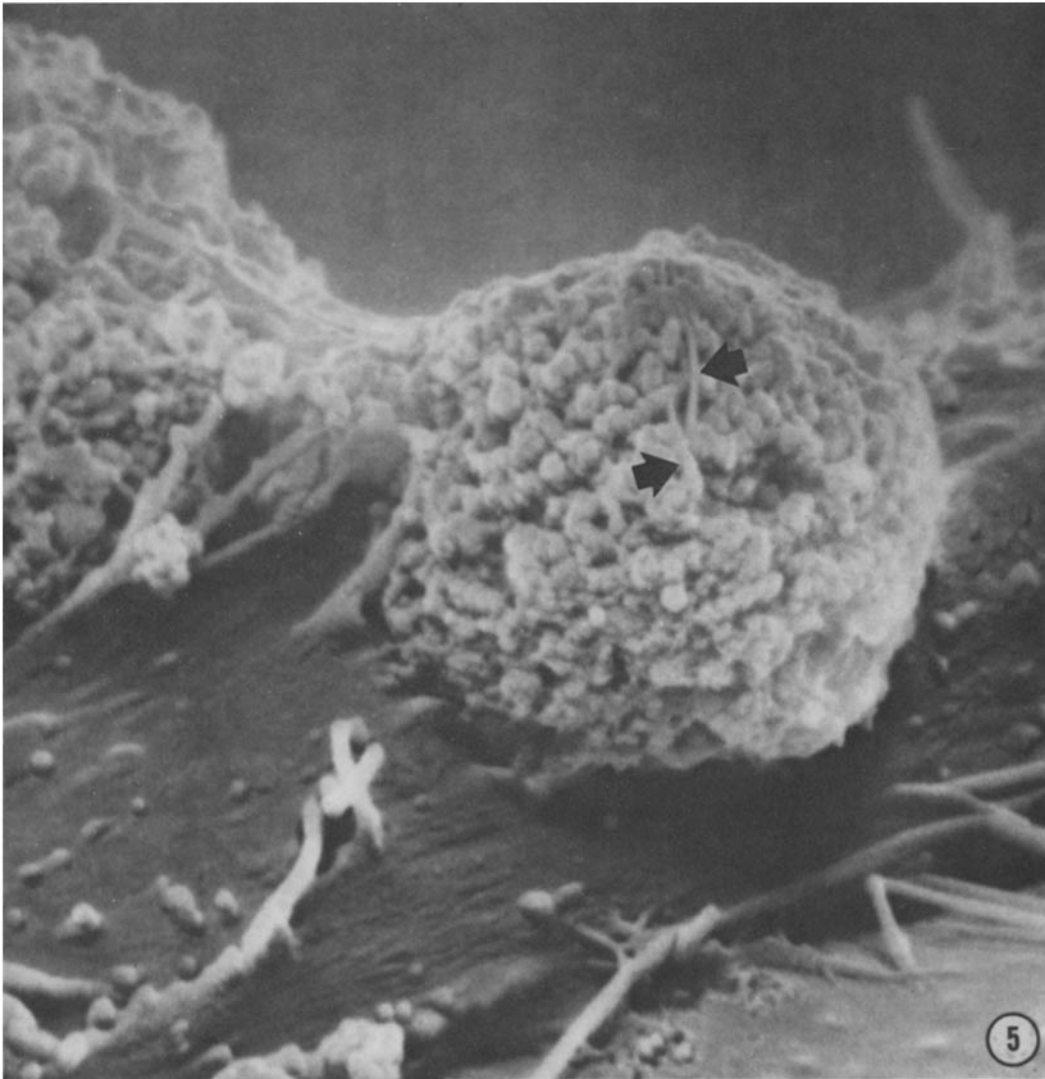


FIG. 5. Scanning electron microscopy of plasmacyte attached to a fibroblast. The irregularities on the surface of the plasmacyte are microvilli (arrows) and protruding vesicles (Fig. 4). The surface of the fibroblast is relatively smooth with scattered short and long microvilli. The points of contact between plasmacyte and fibroblast are indistinct. 10,000, 10 kV, 60° tilt.

the plasmacytes occurred 3 days after conditioned media from other cells was added. It was also noted that the lymphoid cell lines derived from normal donors not only failed to establish in the presence of fibroblasts, but also the addition of FCM resulted in decreased viability of the lymphoid cells.

Characteristics of the Stimulating Factor—Fractions containing plasmacyte-

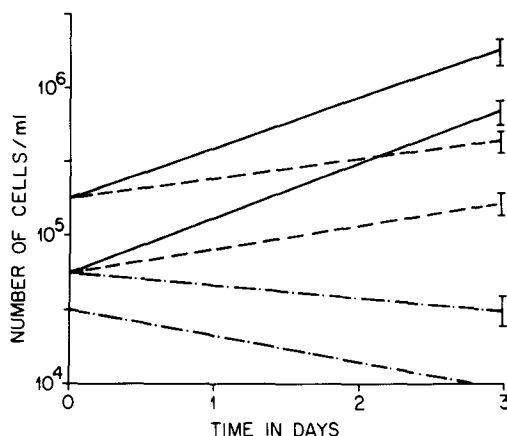


FIG. 6. Growth curves of cultured cells containing fibroblasts (—). Cultures containing 1:16 dilution of FCM (---). Cultures containing media only (— · —). Doubling time in cultures containing fibroblast was every 21–24 h. All cultures containing media only eventually had total cell death. Cultures containing a 1:16 dilution of the FCM could continue to proliferate provided they were fed every 3 days with media containing the factor.

stimulating factor (PSF) were found in tubes 13–17 from the Sephadex G-100 column, and in tubes 18–22 from the Sephadex G-200 column (Fig. 7). The fractions containing bovine albumin from FCS were found to prolong life of the myeloma plasmacytes for 6 days; however, there was no increase in the viable cell count during this period. The fractions containing amino acids kept 10% of the cells viable for 3 days, while the contents of the remaining fractions neither stimulated proliferation nor promoted life. The fractions containing the factor showed a fourfold increase of viable cells during a period of 6 days.

The mol wt of the factor was estimated to be about 150,000 daltons by comparison with the G-200 distribution of proteins of known size. Further purification and characterization of the stimulating factor is in progress.

The factor was found to be heat labile and sensitive to trypsin. The cell counts failed to raise and, in fact, decreased from 1×10^5 to 8×10^4 72 h after the addition of the trypsin-digested PSF. The factor was stable for 3 days at 37°C and for more than 1 yr at -70°C .

DISCUSSION

The results indicated that successful initiation of a human neoplastic plasmacyte line was dependent upon three factors: (a) the ability of the myeloma cells to form colonies in an agar overlay (7, 8); (b) the presence of fibroblastic cells; and (c) high concentrations of amino acids in the culture medium. Similar results have been reported for a murine plasmacytoma using culture medium of tissue macrophages or spleen phagocytic cells (1), and by the use of fibroblastic feeder layers (2, 19).

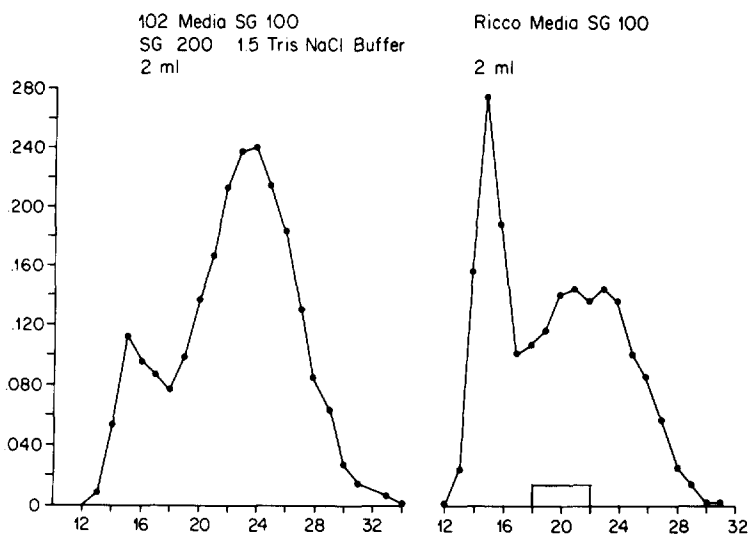


FIG. 7. G-200 chromatography of partially purified media. Conditioned media from a hematopoietic cell line LA-102 (control) and fibroblast cell line (Ricco) was concentrated 10-fold and fractionated on a Sephadex G-100 column equilibrated with 1.5% Tris-NaCl buffer at pH 8.6. Tubes containing protein of 100–200,000 daltons were pooled and refractionated on a Sephadex G-200 column. The above figures represent the protein peaks at 280 OD of fractions 12–34. The PSF was found in tubes 18–22 of the fibroblast culture medium (Ricco), but not in comparable fractions of LA-102 medium.

A PSF from a conditioned media, or the presence of irradiated fibroblast, was found to be essential for the maintenance and further proliferation of this established cell line. The myeloma cells proliferated for 2 mo until the death of fibroblasts, and for 3 days in the presence of fresh FCM. The experimental results presented in this report indicate that proliferation depends upon a factor produced by normal human fibroblasts. The PSF was not required for continuous lymphoid cell lines. Indeed, it appeared that it might be toxic for some cells.

Production of PSF was characteristic of fibroblasts. All four fibroblast cell lines tested produced the factor. The capacity can be lost with extended culture of the fibroblast lines. In contrast to the fibroblast, lymphoid cell lines (five) and epithelioid cultures (two) failed to produce the factor.

The factor itself was found to be a heat labile protein with a mol wt of 150,000. It was stable for 3 days at 37°C and for more than 1 yr at –70°C.

The ultrastructure of LA-49 revealed a large quantity of endoplasmic reticulum in comparison to that seen in cultured lymphoblasts. The mitochondria were large and situated between ergastoplasmic sacs studded with ribosomes. Electron microscopy of the cell line revealed that cultured cells continue to mature from plasmablasts to mature plasmacytes *in vitro*, a characteristic not present in lymphoid cell lines (20). Light microscopy revealed large Mott bodies and nuclear inclusions typical of a myeloma plasmacyte. These abnormal

morphological characteristics of the neoplastic myeloma cell remained after the cell line became established in culture.

The criteria for determining whether a human cell line is derived from normal or malignant cells has been outlined by Belpomme et al. (19). These criteria suggest that LA-49 was derived from the neoplastic cell. The karyotype of LA-49 revealed polyploidy with no common multiple of a chromosome number, while lymphoid cell lines established in our laboratory are either diploid or pseudodiploid. LA-49 not only formed colonies in soft agar, but produced the myeloma protein IgD lambda. The myeloma protein from LA-49 was identical to that synthesized by the patient's tumor.

Since this was the first time in our laboratory that we were able to study the proliferative, maturational, and functional characteristics of a human plasmacyte cell line, it is essential that additional attempts be made to culture other myeloma cell lines in order to expand upon the results stated here. Further characterization of this cell line and its role in immunological disease is in progress.

It is possible that the location (and the attendant destructive lesions) may depend upon the local production of a growth-supporting factor such as that described here. The concept of normal cell (product) and malignant cell interrelation as a necessary condition of tumor growth deserves consideration in multiple myeloma.

SUMMARY

Cell line LA-49, derived from pleural fluid cells of a patient with IgD multiple myeloma, was established in culture and maintained for more than 1 yr. The D-myeloma protein produced in culture was similar to the serum D-myeloma protein in electrophoretic mobility and in delta- and lambda-chain antigens.

The plasma cell tumor culture, LA-49, differed from numerous immunoglobulin-producing B-lymphoblastoid cell lines established in this laboratory in: (a) Morphology (revealing various stages of maturation); (b) type of immunoglobulin produced (IgD vs. IgM, IgG, and/or, rarely, IgA); (c) growth characteristics (requirement of plasmacyte-stimulating factor); and (d) chromosomal features (polyploid vs. pseudodiploid).

A growth factor was needed for cell division and maintenance of culture viability. This factor was supplied readily by irradiated feeder layers of normal human fibroblasts or conditional media from fibroblast cultures. Preliminary characterization of this factor revealed it to be a protein with a mol wt of approximately 150,000 daltons.

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REFERENCES

1. Namba, Y., and M. Hanaoka. 1972. Immunocytology of cultured IgM-forming cells of mouse. *J. Immunol.* **109**:1193.
2. Namba, Y., and M. Hanaoka. 1969. Immunoglobulin synthesis by cultured mouse myeloma cells. *J. Immunol.*, **102**:1486.
3. Horibata, K., and A. Harris. 1970. Mouse myeloma and lymphomas in culture. *Exp. Cell Res.* **60**:61.
4. Moore, G. E. and H. Kitamura. 1966. Cell line derived from patient with myeloma. *N. Y. State J. Med.* **68**:2757.
5. Pettengill, O. S., and G. D. Sorenson. 1967. Murine myeloma cells in suspension culture. *Exp. Cell Res.* **47**:608.
6. Paraf, A., M. A. Moyne, J. F. Duplan, R. Scherrer, M. Stanislawski, M. Bettane, L. Lelievre, P. Rouze, and J. M. Dubert. 1970. Differentiation of mouse plasmacytomas in vitro: two phenotypically stabilized variants of the same cell. *Proc. Natl. Acad. Sci. U. S. A.* **67**:983.
7. Imamura, T., and L. E. Moore. 1968. Ability of human hematopoietic cell lines to form colonies in soft agar. *Proc. Soc. Exp. Biol. Med.* **128**:1179.
8. Coulson, A. 1968. Lymphocyte culture in agar. *Lancet.* **1**:89.
9. Golde, D., and M. Cline. 1972. Identification of colony-stimulating cell in human peripheral blood. *J. Clin. Invest.* **51**:2981.
10. Anderson, D. R. 1966. Ultrastructure of normal and leukemia leukocytes in peripheral blood. *J. Ultrastruct. Res. Suppl.* **9**:1.
11. De Harven, E., B. Clarkson. 1967. Electron microscopic study of human leukemia cells in tissue culture. *Cancer.* **20**:911.
12. Sorenson, G. D. 1964. Electron microscopic observations of bone marrow from patients with multiple myeloma. *Lab. Invest.* **13**:196.
13. Bessis, M. D. 1961. Ultrastructure of lymphoid and plasma cells in relation to globulin and antibody formation. *Lab. Invest.* **10**:1031.
14. Puck, T. 1958. Action of radiation on mammalian cells. III. Relationship between reproductive death and induction of chromosome anomalies by X-irradiation of euploid human cells in vitro. *Proc. Natl. Acad. Sci. U. S. A.* **44**:772.
15. Puck, T. T., and P. Marcus. 1955. A rapid method for viable cell titration and clone production with HeLa cells in T. C. The use of X-irradiated cells to supply conditioning factors. *Proc. Natl. Acad. Sci. U. S. A.* **41**:432.
16. Darlington, C., and L. LaCour. 1960. The handling of chromosomes. George Allen and Unwin, Ltd., London, England.
17. Mellman, W. J. 1965. Human peripheral blood leukocytes cultures. *In Human Chromosome Methodology*, J. J. Yunis, editor. Academic Press, Inc., New York.
18. Price, Z. H. 1973. A three dimensional model of membrane ruffling from transmission and scanning electron microscopy of culture monkey kidney cells (LLCMK₂). *J. Microsc. (Oxf.)*. **95**:493.
19. Belpomme, D., and J. Minowada. 1972. Are some human lymphoblastoid cell lines established from leukemic tissue actually derived from normal leukocytes? *Cancer.* **30**:282.
20. Moore, G. E., H. Kitamura, and S. Toshima. 1968. Morphology of cultured hematopoietic cells. *Cancer.* **2**:425.