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Cloning of Immunoglobulin-Producing Human Leukemic and Lymphoma Cells in Long-Term Cultures.* (31677)

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Immunoglobulin (Ig) production in a large number of *in vitro* cultured human leukemia and Burkitt lymphoma cell lines has recently been reported(1,2,3,4). These studies indicated that the cell lines produced diverse immunoglobulins. Immunofluorescent studies of smears of these cell lines demonstrated that the specific immunoglobulin staining occurred in the cytoplasm of many cells, although there were always some cells which showed no staining. These observations suggested a possible heterogeneity of these cell lines with respect to immunoglobulin production and our cloning studies were undertaken in an effort to elucidate this question.

Very recently a technique for deriving single cell clones from a line of Burkitt lymphoma cells by a semi-solid agar procedure was developed in this laboratory(5). The present communication describes successful cloning of 2 lines of human leukemia cells and 2 lines of Burkitt lymphoma cells and an immunofluorescent analysis of the clonally derived cell strains with respect to their production of heavy chain immunoglobulins.

Materials and methods. The cell lines used in these studies were the 64-10 line derived from a patient with myelogenous leukemia by Iwakata and Grace(6); the LKID line derived from a patient with lymphocytic leukemia by Armstrong(7); two lines designated SL-1 and P3J isolated from African patients with Burkitt lymphoma by Stewart *et al*(8) and Pulvertaft‡ respectively. All lines were carried in this laboratory as stationary suspension cultures in a medium composed of 80% Eagle's minimum essential medium and 20% fetal calf serum plus 20 μg/ml of L-serine and 110 μg/ml of sodium pyruvate. The cell cloning procedure is described in more detail elsewhere(5). The above growth medium was used for the cloning but with the addition of 0.4% and 0.3% of ethanol-ether washed Bacto-agar for the base and the seed layers respectively. Five ml of the base agar was solidified in a plastic petri dish (60 × 15 mm). Two or three days after subculture of the cells in growth medium in a 5-7% CO_2 humidified incubator the cells were dispersed singly by pipetting and diluted with the seed agar to concentrations of 100 cells and 10 cells per ml respectively. Two ml of each dilution were layered onto the base agar in a petri dish. The inoculated dishes were then incubated in the CO_2 incubator.

Within 7 to 10 days after plating, colony formation was observed with all 4 cell lines. The number of colonies was counted under a dissecting microscope. The cloning efficiency of each cell line was 6% for 64-10, 13% for LKID, 21% for SL-1 and 72% for P3J. The cloning efficiency of each cell line was independent of the initial cell density and a dilution effect on the number of colonies formed was observed providing evidence that

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‡ Pulvertaft, R. J. V., unpublished data.

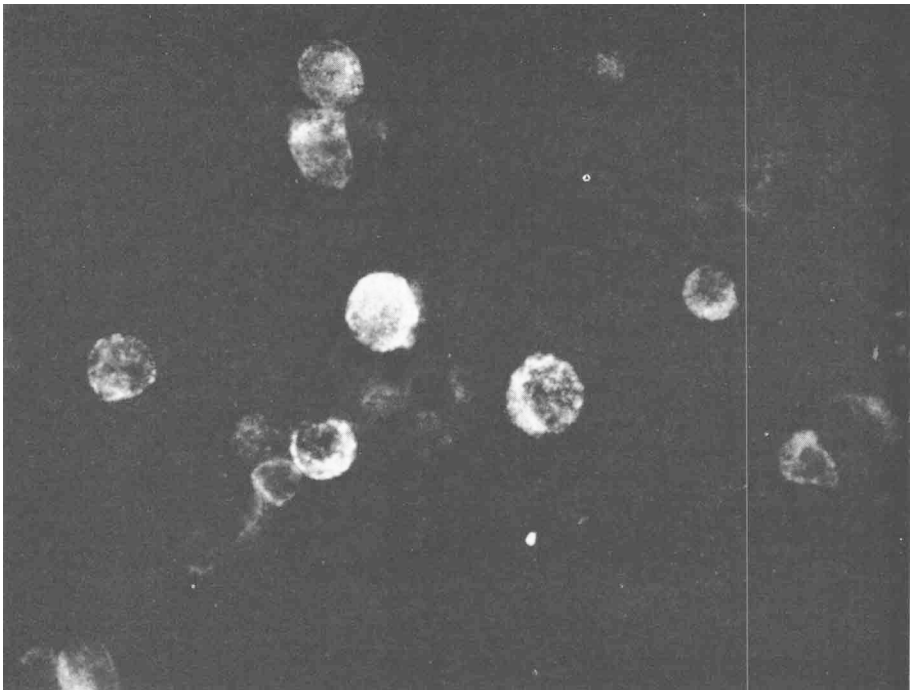

FIG. 1a.

FIG. 1. Immunofluorescent staining of cell clone cultures from the 64-10, LKID and P3J lines (310 × magnification). a. The 64-10 clone no. 6 stained with anti-Ig G. b. The LKID clone no. 11 stained with anti-Ig A. c. The P3J clone no. 5 stained with anti-Ig M.

each colony arose from a single cell. Well isolated colonies were picked up by pasteur pipettes and transferred to growth medium in petri dishes and incubated in the CO_2 incubator. For production of larger quantities of the clonally derived strains the petri dish cultures were transferred to bottles.

Heavy chain immunoglobulin production in the cells of the parent cultures and in the cells of randomly selected clone cultures was determined by immunofluorescence. Fluorescein isothiocyanate conjugated monospecific goat globulin against Ig A, Ig M and Ig G were obtained from Hyland Laboratories (9). The specificities of these reagents were confirmed by immunodiffusion in this laboratory. A 1:20 dilution of each reagent was used for the immunofluorescent studies. Smears were prepared from cells one to three days after subcultivation. They were air-dried and then fixed with acetone for 10 minutes at room temperature. Fixed smears were stained for 30 minutes at 37°C with either anti-Ig G, anti-Ig M or anti-Ig A fluorescein isothiocyanate conjugated goat globulin. The slides were then washed with phosphate buffered saline, mounted and examined

TABLE I. Heavy Chain Immunoglobulin-Production in Clonally Derived Cell Strains as Revealed by Immunofluorescence.*

Cell lines and clones	% † of fluorescent cells stained by Anti-Ig G	Anti-Ig M	Anti-Ig A
64–10 parent	32	0‡	0
64–10 Clone No. 1, 3, 5, 6, 7, 8, 9, 10	23–48	0	0
LKID parent	65	0	68
LKID Clone No. 1, 4, 5, 11	49–72	0	54–81
SL–1 parent	0	38	0
SL–1 Clone No. 1, 2, 3	0	25–56	0
P3J parent	56	62	0
P3J Clone No. 1, 7, 38	0	45–70	0
P3J Clone No. 5, 12	35–68	48–65	0

* The reagents are specific for the heavy chain part of each immunoglobulin.

† Maximum % in more than 2 tests.

‡ A minimum of 2000 cells was examined before classification as negative.

with the light microscope using ultraviolet illumination.

Results. The results are summarized in Table I and Fig. 1. The specific immuno-

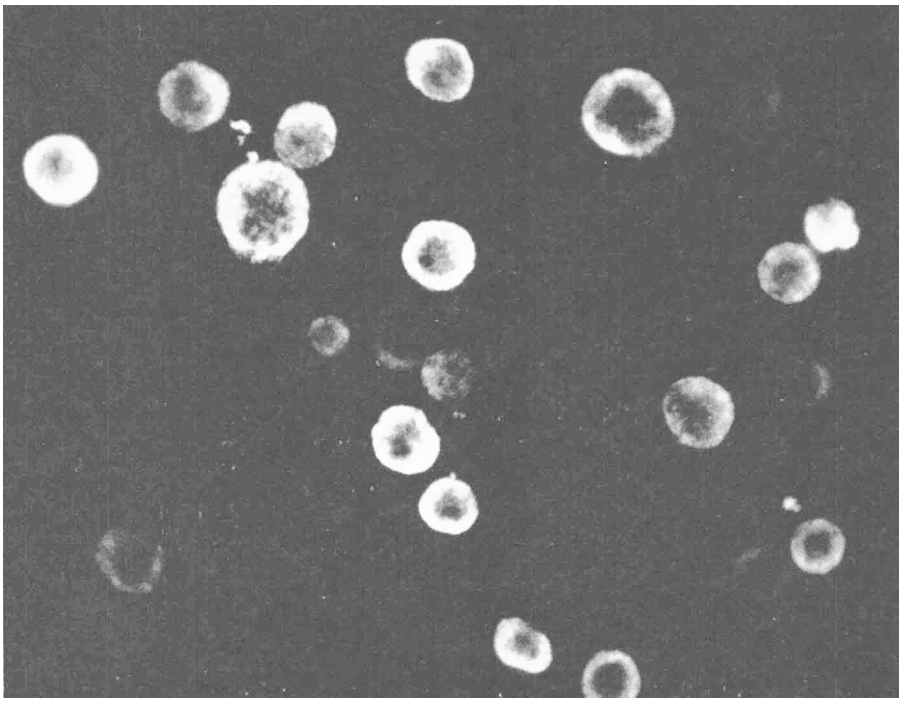

FIG. 1b.

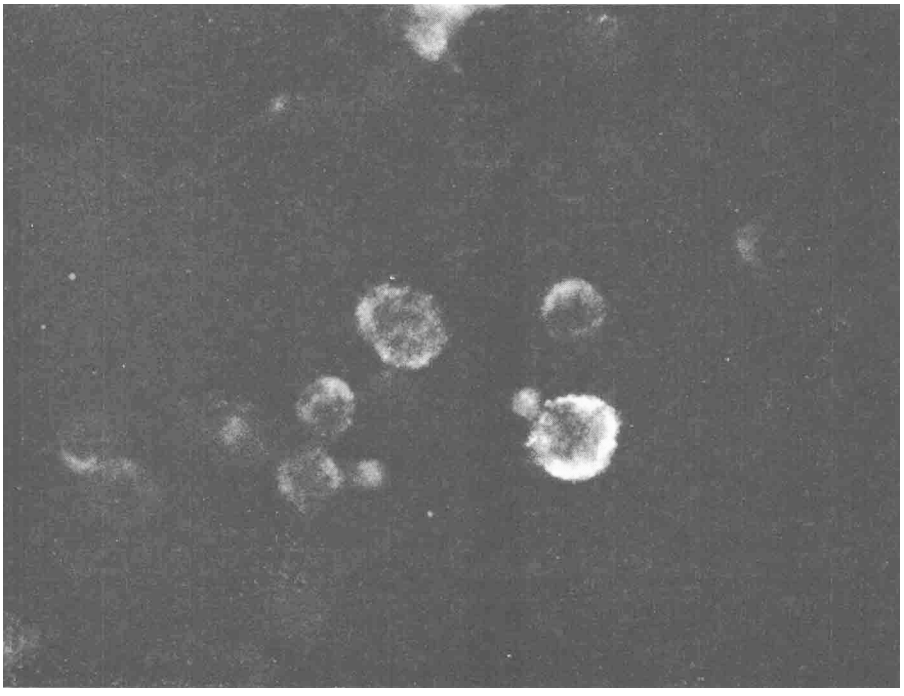

FIG. 1c.

globulin fluorescent staining was evenly distributed in the cytoplasm. No definite nuclear staining was observed. The parent cells and all of the 8 clones of the 64-10 line tested were stained with anti-Ig G and no significant difference was seen between the parent and the clone cultures in terms of numbers of cells staining or brilliance of the fluorescence. With the LKID line all of the 4 clones tested were stained with both anti-Ig G and anti-Ig A, which again was similar to the parent culture. All of the 3 clones tested and the parent culture of the SL-1 line were stained only with anti-Ig M. The cells of the parent culture of the P3J line were stained with both anti-Ig G and anti-Ig M. Two out of 5 clones tested in this cell line were stained with both anti-Ig G and anti-Ig M. However, the remaining three clones were stained only with anti-Ig M and not with anti-Ig G.

Discussion. It is interesting that all of the 20 clones from the 4 cell lines tested were positive with one or two of the 3 reagents used and no negative clones were derived. This suggests that all or most of the cells in the parent cultures of the 4 cell lines possessed the potential to produce some heavy chain component of immunoglobulins. The number of stained cells in each clone culture varied between 20 to 80% which was comparable to their corresponding parent cell cultures. The intensity of the fluorescence and the percentage of fluorescent cells varied from culture to culture probably indicating asynchronous production of heavy chain immunoglobulins in both the parent and clonal lines. It is possible that the unstained cells were capable of heavy chain production but that these cells were studied at a time in their cell growth cycle that they were not producing it.

Four clonally derived strains from the LKID line and 3 strains from the P3J line showed production of both Ig G and Ig A and of both Ig G and Ig M respectively. This indicated the production of two different types of heavy chain immunoglobulins in clones which were derived from single cells. Tanigaki *et al*‖ demonstrated that single cells in the LKID line were stained with both anti-Ig A labelled with fluorescein isothiocyanate and anti-Ig G labelled with tetramethyl rhodamine isothiocyanate. These results with established human cell lines, together with studies by others(10,11), strongly suggest that at least 2 different immunoglobulins may be produced by a single cell.

Cells from the parent culture of the P3J line may be divided into 2 types as a result of the clonal studies; one cell type which produces only Ig M and a second cell type which produces both Ig M and Ig G. Thus it appears that the parent P3J culture contained at least two types of cells while the other 3 cell lines showed no evidence of cell heterogeneity as measured by these parameters.

Summary. Single cell clones were derived from 4 lines of human leukemia and Burkitt lymphoma cells in long-term culture by a semi-solid agar procedure. Some clonally derived cell strains were shown to produce only one component of heavy chain immunoglobulins and others produced 2 components as determined by immunofluorescence. One of 4 cell lines tested contained 2 different types of cells. One cell type produced only Ig M. The other cell type produced both Ig M and Ig G. All of the clones derived from 3 other cell lines produced the same immunoglobulins as their parent cultures.

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Distribution of Radioactivity in Body Fat and Organs of Rats Treated with Labeled Quinestrol, Ethynylestradiol or 17β-Estradiol. (31678)

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Ethynylestradiol - 3 - cyclopentyl ether (EECPE, quinestrol) differs from its parent compound, ethynylestradiol, (EE) in several respects. Orally administered EECPE is stored unaltered in perirenal fat and brain of rats whereas EE is not(1-3). Unlike EE and most other steroids, EECPE is absorbed in significant amounts through the lymphatic system when administered orally in a lipid vehicle(4). An oil rather than aqueous vehicle also enhances storage of EECPE in both perirenal fat and brain whereas little or no EE is stored in fat or brain regardless of vehicle(3). Absorption of intraduodenally administered EECPE but not EE from an oily solution is prevented by excluding bile and pancreatic juice from the small intestine (3).

The peculiar affinity of EECPE for perirenal fat and brain and the unexplained influence of a lipid vehicle on absorption, transport and storage of this compound prompted further studies. The present communication describes the distribution of radioactivity in different types of body fat, in various areas of the brain and in major organs of rats treated with doubly-labeled (^3H and ^{14}C) EECPE in comparison with that obtained using tritiated EE or 17 β-estradiol (E_2).

Materials and methods. Preparation of radioactive steroid doses. Ethynylestradiol-6,7-^{3}H-3-cyclopentyl ether* (specific activity 0.78 μc/μg) and ethynylestradiol-3-cyclopentyl-1-^{14}C ether* (specific activity 0.0392 μc/μg) were mixed to give specific activities of approximately 0.26 μc ^3H and 0.026 μc ^{14}C per μg EECPE and thus a $^3H/^{14}C$ ratio of 10:1. Ethynylestradiol-6,7-^{3}H* (specific activity 0.93 μc/μg) and 17 β-estradiol-6,7-^{3}H† (specific activity 140 μc/μg) were diluted with the corresponding unlabeled steroids to give similar specific activities (≅ 0.26 μc ^3H/μg).

Radioactive steroids were dissolved in sesame oil or suspended in an aqueous vehicle (0.4% Tween 80, 0.9% benzyl alcohol, 0.9% NaCl, 0.5% carboxymethylcellulose in distilled water). For the distribution studies, doses were approximately 19.1 μg (5 μc ^3H) and in the case of EECPE 5 μc ^3H and 0.5 μc ^{14}C. Actual doses were always determined by liquid scintillation spectrometry and when applicable data were corrected to a constant dose of 5.0 μc ^3H(3). In the studies of distribution of EECPE in brain, a constant dose of 13.8 μc ^3H/20 μg/0.5 ml suspending vehicle (no ^{14}C) was used.

Experimental procedures. (1) *Distribution studies.* Female rats (Hemlock Hollow) weighing 280-300 g were used. One month after bilateral ovariectomy the animals were divided into 3 groups and dosed orally with labeled EECPE or EE or subcutaneously with labeled E_2 in suspending vehicle. The rats were placed in metabolic cages and urine and feces were collected. Twenty-four hours after dosing, the rats were anesthetized with ether and exsanguinated by heart puncture. In addition to plasma and red cells, the following tissues and organs were collected and

* Synthesized by Mr. E. Merrill, radiation officer, Warner-Lambert Research Institute. The steroids were judged radiochemically pure by thin layer chromatography.

† New England Nuclear Corp. Lot. No. 134-193-105. About 90% radiochemically pure by thin layer chromatography.