Presence of oncornavirus-like particles in the P388 murine leukemic cell line

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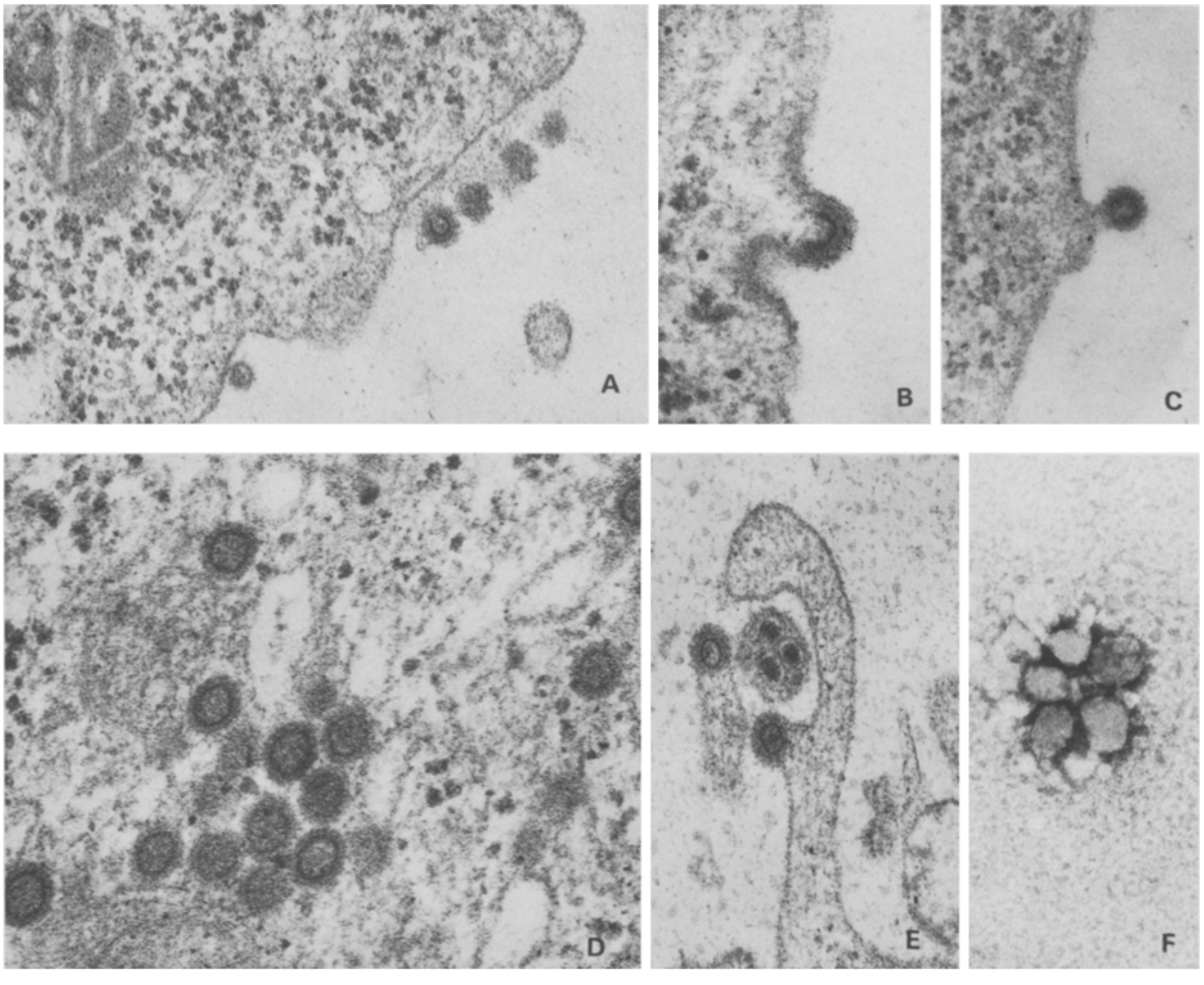
Summary. A culture of P388 murine lymphoblastoid cells has been shown to contain type C oncornavirus-like particles budding at the plasma membrane. Occasionally intracytoplasmic type A and immature type B particles were also observed by electron microscope techniques. The discovery of oncornavirus-like particles in the P388 cell line increases the utility of this neoplastic system for detecting potential antineoplastic agents.

A culture of P388 murine lymphoblastoid cells obtained from the National Cancer Institute's program at Arthur D. Little Co. is being used for in vitro cytotoxicity evaluation of biosynthetic antineoplastic agents in our laboratories. Because of possible biohazard and need for more detailed knowledge of these cells, it was necessary to determine if this cultured cell line produces virus-like particles.

After several serial in vitro passages in our laboratory, a sample of P388 cells was prepared for ultrastructural examination with a transmission electron microscope. A 50 ml culture was inoculated at a density of 5×10^4 cells/ml and grown in Fischer's medium (Grand Island Biological Co.) with 10% horse serum (v/v), 100 U penicillin and 100 µg streptomycin per ml without agitation for 48 h at 37 °C. The final cell density was 6.6×10^5 cells/ml. The culture was not exposed to known chemical inducers of oncornaviruses. A 20 ml sample was centrifuged 5 min at $800 \times g$, prefixed with 1.5% glutaraldehyde, washed thoroughly with modified Sorenson's buffer[4], and fixed with 1% osmic acid for 1 h. After dehydration with ethyl alcohol and propylene oxide, the P388 cells were embedded in Epon and cured. Thin sections were obtained with a diamond knife, stained with 1% uranyl acetate and 2% lead citrate[5], and observed with a Philips 300 transmission electron microscope operated at 80 kV.

Almost every sectioned P388 cell observed had type C oncornavirus-like particles budding at the plasma membrane (figure, A, B and C), or occasionally into an intracytoplasmic vesicle. Type C particles that were apparently mature were frequently observed along the outside of the



A, *B*, and *C* P388 cell peripheries with budding type C particles. *A* × 64,800; *B* × 116,000; *C* × 55,000; *D* Intracytoplasmic type A particles in P388 cultured cells × 113,000; *E* Type A particles associated with microvilli, × 70,000; *F* Leukemia virus-like particles concentrated from cell-free supernatant of a P388 cell culture, 1% potassium phosphotungstate stain, × 73,000.

plasma membrane. Intracytoplasmic type A particles were occasionally seen. Several P388 cells were observed to have dense aggregates of type A particles in their cytoplasm (figure, D). In a few instances immature type B particles or apparent budding of type A particles were seen (figure, E). Cell-free particulate material was harvested from the supernatant of another 48 h P388 culture by differential centrifugation, exposed to 1% glutaraldehyde in a tris-sodium chloride-ethylenediaminetetraacetic acid buffer[6], and then subjected to negative staining with 1% potassium phosphotungstate. Numerous leukemia virus-like particles, lacking distinctive peplomers or spikes, were observed (figure, F). Type C particles seem to be the predominant oncornavirus-like entities produced by cultured P388 cells. The production of type C particles is apparently a common event in the cultured P388 cells to be so readily detectible by the relatively insensitive electron microscopy approach. Further experiments to assay in vitro infectivity titers of P388 cells and their type C particles in other cultured cells will be undertaken.

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Identification of lymphocyte subpopulations by simultaneous E-rosette formation and unspecific acid esterase staining

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Summary. E-rosettes of human peripheral blood lymphocytes were stained by an unspecific acid esterase stain; 74.5% of the rosette forming cells were esterase positive, while only 30% of the non-rosette forming cells showed a reaction product.

There is accumulating evidence that lymphocytes presenting with localized acid alpha-naphthyl-acetate-esterase (ANAE) activity are T cells. ANAE positive lymphocytes are found mainly in the cortical areas of the mouse lymph node, but not in the germinal centres[1]. Removal of E-rosette forming lymphocytes by Ficoll-Hypaque centrifugation results in a marked depletion of ANAE positive cells in the supernatant[2]. 85% of peripheral human blood lymphocytes and 90% of tonsillar T lymphocytes are ANAE positive, whereas only 12% of isolated B cells are positive[3]. Such cells are characterized by a strong, well-defined and dot-like reaction product, in contrast to human thymocytes, of which only about 30% are ANAE positive, with a faint cytoplasmatic staining of variable intensity. These different staining patterns are described as T-like and Thy-like, as opposed to the well-known intense and diffuse reaction product in monocytes (M-like)[3]. We report further evidence that esterase positive cells are of T cell origin by direct esterase-staining of rosette forming cells.

Methods. Peripheral blood lymphocytes were obtained from 19 healthy volunteers (age range 29–50 years, median 35 years). Ficoll-Ronpacon density gradient isolated lymphocytes were incubated in 0.83% NH_4Cl at 37 °C for 15 min to destroy remaining erythrocytes, washed 3 times with MEM and resuspended in MEM at a concentration of 10×10^6 cells/ml.

Sheep blood was diluted 1:2 with Alsevers solution. Aliquots were washed 3 times with 0.9% NaCl and resuspended at a final concentration of 80×10^6 sheep red blood cells (SRBC)/ml. Anti-SRBC antibodies were removed by incubation of SRBC in bovine serum for 30 min at 4 °C and at 37 °C. 0.05 ml of SRBC free bovine serum and 0.05 ml of the resuspended SRBC were added to 0.1 ml of human peripheral blood lymphocytes. This mixture was centifuged at $200 \times g$ for 5 min, gently resuspended and rosettes (≥ 3 SRBC/lymphocyte) counted in an aliquot of this suspension. Thereafter the rosettes were fixed with 5% glutaraldehyde in saline. Smears were prepared by cytocentrifuge sedimentation and immediately stained for 16 h at 4 °C by the method described by Müller[1] using alpha-naphthyl-acetate as substrate and hexazotized pararosanilin as coupler. We defined as 'positive' all lymphocytes showing some kind of reaction product.

Subgroups of the 'positive' population were classified as 'localized': cells displaying a strong, dot-like staining pattern with 1 or 2 dots (T-like); as 'granular': cells displaying a more diffuse, fine granular staining pattern in the cytoplasm (Thy-like).

Cells were called 'negative' if they did not show any evidence of ANAE activity.

Results and discussion. In preliminary experiments we had determined in parallel the percentage of ANAE positive and E-rosette forming cells in the same blood sample of 25 healthy volunteers: $94 \pm 4.2\%$ of the blood lymphocytes were ANAE positive and $75 \pm 4.7\%$ formed E-rosettes.

In these series we directly stained E-rosettes as shown in the figure. The intense dot-like reaction product can be seen clearly, the characteristic localization being at the periphery of the cell.

The results are shown in the table: 74.5% of the ANAE positive cells formed E-rosettes, 25.5% did not. Of the 'localized' subgroup 76%, of the 'granular' subgroup 66%, formed E-rosettes, whereas only 30% of the ANAE negative lymphocytes did so. These results add further evidence that ANAE positive cells are in fact T cells. The question remains, however, why 25.5% of ANAE positive cells did

Percentages of E-rosette positive and negative lymphocytes among the esterase subpopulations

	E-rosettes Positive (73%)	Negative (27%)
Esterase positive	74.5± 8.8	25.5± 8.8
Localized	76 ± 8.1	24 ± 8.1
Granular	66 ±24.9	34 ±24.9
Esterase negative	30 ±20.4	70 ±20.4