A Human Leukocyte Culture with an Unusual Cytoplasmic Envelopment of Herpes-type Particles


SUMMARY

A leukocyte culture, G-6, was established from a pretreatment sample of peripheral blood from a 33-year-old patient with chronic myelogenous leukemia. When studied under time-lapse cinematography, G-6 cells exhibited great surface activity, not observed in other cultured leukocyte cells. A majority of G-6 cells had 45 chromosomes. Herpes-type particles in this culture acquired their envelopes in the cytoplasm of infected cells in a manner not previously reported in any of the established human leukocyte cultures.

INTRODUCTION

Cell cultures have been established from the peripheral blood of normal (1, 2, 8, 12) and leukemic (1-5, 13, 25) human subjects and also from hematopoietic tissues (7) and tumors (7, 9, 18, 19) from patients with leukemia and lymphomas. Although most of these cultures contain HTV particles, the relationship of these particles to leukemia is unknown. This communication describes the establishment of a cell line from a pretreatment sample of peripheral blood of a patient with chronic myelogenous leukemia and an unusual cytoplasmic envelopment of herpes-type particles in infected cells of this culture.

MATERIALS AND METHODS

Case Report. A 33-year-old man (RH No. 34-06-55), employed as an X-ray technician for the preceding 5 years, was admitted on June 11, 1968, with 2 days of progressive left-upper-quadrant abdominal pain. His only prior illness was a genitourinary tract illness treated with tetracycline 3 weeks prior to admission. On physical examination, there was marked abdominal tenderness, maximal in the left upper quadrant. The spleen was enlarged to the iliac crest. There was no lymphadenopathy. The white blood cell count was 66,000/cu mm, with 39% polymorphonuclear forms, 10% nonsegmented forms, 9% metamyelocytes, 3% myelocytes, 12% promyelocytes, 17% blasts, 9% basophils, and 1% lymphocytes. There were 6 nucleated red blood cells/100 white cells. The platelet count was 69,000/cu mm. Uric acid was 8.3 mg/100 ml. Bone marrow aspiration was repeatedly unsuccessful. Leukocyte alkaline phosphatase was markedly decreased. A diagnosis of chronic myelogenous leukemia was made. In the hospital, signs of an acute abdominal condition developed. Extensive splenic infarction was suspected, and splenectomy was performed. The massively enlarged spleen (2.2 kg) showed multiple small infarcts and extramedullary hematopoiesis. Liver biopsy showed extensive immature granulocytic infiltration and many eosinophils. Busulfan and allopurinol were started. The white blood cell count rose to 340,000/cu mm but subsequently fell to 7,500/cu mm. The differential count was not greatly altered. The patient continued to require transfusions, and the platelet count remained low at discharge after 7 weeks of hospitalization.

His 2nd admission was 11 days later, with sore throat and fever. The hematocrit was 22% and the white blood cell count was 66,000/cu mm, with 9% polymorphonuclear forms, 9% nonsegmented forms, 3% metamyelocytes, 39% myelocytes, 12% promyelocytes, and 20% blasts. The platelet count was 26,000/cu mm. Bone marrow aspirations were again unsuccessful. Considered to be enduring a blast crisis of chronic myelogenous leukemia, he was treated with vincristine, prednisone, 6-mercaptopurine, and methotrexate. The white blood cell count fell to 22,000, and the platelet count fell to 6,000/cu mm. Platelet transfusions were administered, but the patient died 1 week after admission on August 20, 1968.

Autopsy revealed extensive leukemic infiltration of all organs and some fibrosis of marrow. The final diagnosis was myelofibrosis progressing to granulocytic leukemia. The immediate cause of death was subarachnoid hemorrhage.

Establishment of Cell Line. Red blood cells from a sample of heparinized blood freshly drawn at the time of the patient's 1st admission were allowed to settle by gravitation for 1.5 hr. The leukocyte-rich plasma was separated and passed through a layer of Pyrex glass wool fiber No. 3950 at the rate of 30 drops/min to remove large phagocytic cells. The glass wool was washed twice with RPMI 1629 medium supplemented with 25% fetal calf serum. The filtered plasma was spun down at 800 rpm for 10 min, and the cells were resuspended in fresh medium so as to provide approximately 2 X 10^6 cells/ml.
Two-oz glass bottles were used to initiate and establish a suspension culture of leukocytes, herein designated as G-6.

**Chromosome Studies.** Cells from G-6 culture were processed for chromosome analysis according to the modified technique (10) of Moorehead et al. (15).

**Electron Microscopy.** Techniques used for electron microscopic examination of tissues have been described elsewhere (1, 2).

## RESULTS

**Peripheral Blood.** The peripheral blood of the patient at the time of his 1st admission contained approximately 15 to 20% abnormal primitive cells (Fig. 1) among mature, nonsegmented granulocytes. The abnormal cells appeared larger than the abnormal primitive cells (Fig. 1) among mature, nonsegmented time of his 1st admission contained approximately 15 to 20% of Moorehead effl. (15).

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**RESULTS**

**Peripheral Blood.** The peripheral blood of the patient at the time of his 1st admission contained approximately 15 to 20% abnormal primitive cells (Fig. 1) among mature, nonsegmented granulocytes. The abnormal cells appeared larger than the other leukocytes and had large nuclei, sometimes lobulated, which filled almost the entire volume of the cells (Fig. 2). The nucleoli of these cells were prominent, and the chromatin material appeared finely reticulated. Such cells had a thin rim of basophilic cytoplasm and were considered to be blast forms.

**Cellular Proliferation and Morphology in Culture.** Approximately 2 weeks after initiation of the culture, some clumps and giant cells (Fig. 3) appeared. The culture was split in 2 and fed with fresh medium every week for the following 3 weeks. In the beginning, the cells grew very slowly, but they entered a logarithmic phase in 3 months when the generation time was approximately 24 hr. Thereafter, the culture was divided 2 or 3 times a week when the cell count was approximately 2 × 10⁶/ml. The cells grew in suspension and, on staining, resembled primitive blast cells. Giant cells were not seen 5 or 6 weeks after initiations of the culture. Cultured cells showed great surface activity, as observed in time-lapse cinematography (Fig. 4). For such a study, 3 separate runs were made during a 3-month period. The duration of cinematography varied from 24 to 48 hr. Cells were grown in RPMI 1629 medium containing 20% fetal calf serum in a Sykes-Moore chamber at concentrations ranging from 1 × 10³ to 1 × 10⁵ cells/ml. Temperature around the chamber was maintained at 37°, and 30 to 40 cells could be seen in the field of view of the camera. Approximately 70% of the cells exhibited surface activity. Other HTV-producing lymphoblast cultures, for example, P-3J (1, 4) and F-132 (1), showed only a few pseudopodia (personal unpublished observations).

**Chromosome Studies.** The modal number of this culture appears to be 45 (Table 1). There is monosomy of 1 chromosome of Group C (Chromosomes 6 to 12) in the size range of No. 7 or 8. Concomitant with this is the loss of 1 from Group G and the appearance of a Group C about the size of an X chromosome. This may represent the translocation of all or part of a G chromosome to one of Group C. No aberrations of chromosomes were observed, and the incidence of polyploidy was less than 2%.

**Electron Microscopy.** Electron microscopic examination of the peripheral blood leukocytes showed that cells that could be considered blast forms by hematological examination were not significantly larger than the more mature leukocytes. The former had multilobulated or deeply indented nuclei (Fig. 5) with no projections of nuclear envelope. Cytoplasmic organelles were few, and the ground substance was filled with ribonucleoprotein granules. No virus-like particles were observed in the original sample of blood.

Cells from the G-6 culture exhibit the characteristic morphology of lymphoblastoid cells, which has been described previously by a number of investigators (4–6, 8, 14) and will not be repeated here. Our comments will be confined to a description of the phases of virus development. In early stages of virus synthesis, nuclei of virus-synthesizing cells contain “empty” and nucleated particles (Fig. 6). The nucleoid, or core, is either a centrally located, dense mass or a hollow, spherical shell (Fig. 6, arrows). In the latter case, the virus particles appear as double-shelled, spherical bodies in thin-sectioned preparations.

The cytoplasm of infected G-6 cells contains some “naked” virus particles and some virions enclosed, either partially or completely, by closed membranous sacs (Figs. 7 to 10). A majority of these particles, whether naked or enveloped, are nucleated, with the dense central core exhibiting a variable structure or morphology (Figs. 7 and 8). Virus particles with hollow spherical cores, similar to those seen in the nuclei, are not observed within the cytoplasm. The membranous sacs can occasionally enclose 2 or 3 particles (Fig. 10). The morphological characteristics of these sacs are quite different from those of any other membranous component of the cytoplasm. Whereas the outer surface of the sacs appears to be smooth, the inner one shows the presence of fringes (Fig. 10). In partially enclosed or enveloped virus particles, the membranous sacs are separated by an electron-lucent material approximately 20 μ thick; in completely enveloped particles, either the fringes on the inner surface of the sac increase considerably in number or the material within the sac becomes less electron-lucent, with the result that the various components of the sac become indistinguishable (Figs. 8 and 9, D). There is no evidence from a close examination of the electron micrographs (Figs. 7 to 9) that the greater density of a few envelopes (Figs. 8 and 9, D) is due to a different plane of

<table>
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<th>Chromosome counts of cultured G-6 cells</th>
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sectioning of the enveloped particles. The various forms of enveloped virus particles observed in the cytoplasm of an infected G-6 cell can represent different stages either in the maturation of herpes-type particles or in the degradation of virions reabsorbed from the culture fluid (see "Discussion"), since the extracellular particles have the morphology of cytoplasmic particles with a dense envelope around them.

Infected cells in G-6 culture also contain another form of enveloped herpes-type particles. Such particles, only occasionally seen, occur within the cisternae of rough ER (Figs. 11 to 13, unlabeled arrows) and have a single membrane shell as the envelope. These enveloped particles measure approximately 140 mμ in diameter. Spherical bodies with the morphological details of these single-shell envelopes are occasionally observed within a nucleus, but these do not contain any particle (Figs. 11 and 12, heavy arrows). Because of the rare occurrence of such enveloped particles, it could not be determined whether the single-shell envelope was derived from nuclear membrane or ER or was synthesized de novo in the nucleus.

G-6 cells synthesizing herpes-type particles contain double-shelled, spherical bodies, which appear in thin sections as doughnut shaped (Figs. 11 to 13, X). Such bodies occur within the dilations of nuclear envelope. The presence of incomplete forms of these structures (Fig. 12) suggests that they arise from reduplication of the nuclear membrane and are not any kind of virus particles.

DISCUSSION

The enveloped HTV particle observed in the cytoplasm of infected G-6 cells can represent either newly synthesized virions undergoing a process of maturation or reabsorbed virions undergoing a degradative process. Although electron micrographs alone cannot distinguish between the 2 processes, events in the infection of cells by similar viruses are well known and should help one arrive at a correct interpretation of the static pictures. It has been shown by Morgan et al. (16) that the 1st stage during the uptake of the HSV by a HeLa cell is the attachment of the viral envelope to the plasma membrane of the host cell. At about 10 min post-viral inoculation, naked virions were observed in the cytoplasm of the HeLa cells. Between 20 and 40 min, viral capsids were observed in various stages of disintegration, and cores of HSV were found in the cytoplasm. When changes in the nuclear matrix were first observed at 4 hr postinoculation, no virus particle was ever seen in the cytoplasm (17). If the virus particles in the G-6 culture belong to the herpes group of viruses, the enveloped virus particles observed in the cytoplasm of infected cells cannot be considered to be those reabsorbed from the culture fluid for initiating infection of the cell in question. They can, however, be phagocytized by the cell. If the lymphoblastoid cells in the G-6 culture or in other human leukocyte cultures were phagocytic, phagocytosis of HTV by cells of such cultures should have been observed by different investigators. In the absence of such an observation in the G-6 culture, it is reasonable to believe that the various stages in the envelopment of HTV observed in cells from this culture represent cytoplasmic maturation of virus particles.

The envelopment of the HTV particles in human leukocyte cultures has been reported to occur at different sites. Virus particles associated with the Burkitt's lymphoma culture SL-1 acquire their envelopes from the nuclear membrane (4), whereas those associated with EB-1 (7), Q1MR-AMB/1, Q1MR-AMB/2 (18), and B-35-M (23) cultures acquire them from cytoplasmic membranes. In other cultures, both nuclear and cytoplasmic envelopment of the particles has been suggested (4). In the G-6 culture, herpes-type particles appeared to become enclosed by membranous sacs in the cytoplasm. The interior of these sacs appeared to darken in forming the envelope. While this process of cytoplasmic envelopment has not been previously described for the HTV present in other leukocyte cultures, similar observations have been made on the envelopment of the HSV (11, 17, 20, 21, 22). In these studies, rows of virus particles aligned in membranes or in vesicular sacs (similar to Fig. 10) have been reported. In some instances, these membranes were considered to be of nuclear origin although they occurred in cytoplasm (17, 21). In other studies, the membranes were thought to be of cytoplasmic derivation, but it was not certain whether they were a component of the Golgi apparatus or smooth ER (11, 22). Although various stages in the engulfment of particles by closed membranous sacs are observed in G-6 cells, the nature and origin of these sacs are unknown. In G-6 cells, the Golgi apparatus and the smooth and rough ER are scant; it cannot therefore be definitely stated that the membranous sacs enveloping HTV are derived from these organelles. The possibility that the membranous sacs are synthesized de novo by the infected cell cannot be ruled out, since they are not found in non-virus-producing cells.

The derivation of the single-shell envelope of HTV particles occurring within the cisternae of rough ER or within the nuclear envelope is speculative. Virus particles could acquire such an envelope either from the inner nuclear membrane or from the ER. Since it is known (24) that the outer nuclear membrane of a cell could be continuous with the ER, virus particles acquiring their envelopes from the inner nuclear membrane could be observed within the cisternae of ER. Alternatively, it is also possible that naked HTV particles occurring free in the cytoplasm acquire the single-shell envelopes by a process of budding from the lamellae into the cisternae of ER. If the particles acquire their envelopes by either of these processes, the significance of spherical bodies having the same size as the envelopes and occurring free in the nucleus is not known.

ACKNOWLEDGMENTS

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REFERENCES

1. Chandra, S., Liszczak, T., and Monroe, J. H. Small Particulate Debris Adhering to Cell Surfaces in Human Leukocyte Cultures:

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Figs. 1 and 2. Photomicrographs of a smear of peripheral blood taken at the time of the patient's 1st admission. Few blast cells (arrows) with large, lobulated nuclei and only a rim of basophilic cytoplasm are seen. Fig. 1, × 300; Fig. 2, × 1,200.

Fig. 3. Photomicrograph showing the presence of giant cells approximately 2 weeks after initiation of the culture. × 100.

Fig. 4. Cinematograph of G-6 cells exhibiting great surface activity in culture, as evidenced by long surface projections (arrows). × 380.

Fig. 5. Low-power electron micrograph of a small area from a section of a pellet ofuffy coat from the same sample of blood as in Figs. 1 and 2, showing "blast" cells with large, lobulated nuclei. × 7,200.

Fig. 6. Portion of an infected G-6 cell synthesizing HTV particles in the nucleus. Both empty and nucleated forms (arrows) of particles are seen in the nucleus. The nucleoid is either a dense mass or a hollow, spherical shell (inset, arrows). The cytoplasmic ground substance is scant and only a few elements of ER are seen. Mitochondria have a beaded structure. × 15,000; inset, × 50,000.

Fig. 7. A small area of Fig. 6 at a higher magnification. A few HTV's appear enveloped, either partly or completely, by close membranous sacs ( arrows), while others ( heavy arrows) are free in the cytoplasm. × 50,000.

Fig. 8. Area similar to that shown in Fig. 7 from another infected G-6 cell exhibiting virus particles in different stages of envelopment by closed membranous sacs ( arrows). Heavy arrows, particles free in the cytoplasm. D. particles in which the various components of envelopes are indistinguishable. × 30,000.

Fig. 9. Three herpes-type particles in different stages of envelopment in the same cell as in Fig. 8. D. particles in which the various components of envelopes are indistinguishable. × 30,000.

Fig. 10. Electron micrograph of 3 herpes-type particles partially engulfed by a closed membranous sac. × 100,000.

Figs. 11 to 13. Infected G-6 cell showing the presence of HTV enveloped by a single shell and occurring within the nuclear envelope and the cisternae of ER ( arrows). X, various profiles resulting from reduplication of nuclear membrane. Single-shelled, spherical bodies ( heavy arrows) are observed within the nucleus. Three naked herpes-type particles are seen within the nucleus. Fig. 11, × 20,000; Fig. 12, × 50,000; Fig. 13, × 100,000.
Cytoplasmic Envelopment of HTV

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