BRIEF REPORT

The Isolation of Cytomegalovirus from Peripheral Blood

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DURING A STUDY designed to detect the presence of transforming agents in the blood of patients with leukemia, a cytomegalovirus was isolated from one patient. The essence of the series of experiments was to co-cultivate leukemic cells and human embryonic fibroblasts and to maintain these latter for a long period of time to observe any evidence of transformation. In none of these experiments, except the one to be considered here, is there any evidence so far to suggest the presence of virus; nor is there any evidence of transformation.

The patient was a boy, aged 8 years, in whom acute lymphatic leukemia was first diagnosed in May 1965. By the time the first blood sample was taken on June 1, 1966, the child had been treated with steroids, 6-mercaptopurine, methotrexate, cyclophosphamide, and X-irradiation. Vincristine was used between the first and second blood samples. The child died on July 21, 1966, one day after the second blood sample was taken. The family history is of some interest: The mother had an X-ray pelvimetry during the pregnancy and the child was delivered by Caesarian section. An older brother is alive and well, but a younger sister has a neuroblastoma.

Blood was taken into heparin, spun, and the buffy coat removed; since the white count was low, the buffy coat also included some red cells. This suspension of red and white cells was then washed three times in tissue culture medium (medium F10, 80 per cent; agamma bovine serum, 10 per cent; and tryptose phosphate broth, 10 per cent). A suspension of low-passage human embryo lung fibroblasts was prepared and half of the suspension mixed with the washed blood cells. Four dishes (with approx. 10⁶ fibroblasts) were then plated out from the mixed suspension and four from the unmixed suspension. The medium was changed the following day. No cytopathic effect was noted in the treated cultures, but they did remain inactive for a few days. Controls and treated cultures were routinely medium changed and subcultured. Four weeks later small foci of abnormal cells were noted in the cultures treated with leukemic cells, and with time these became more extensive. The control plates were consistently negative in all observations. Attempts to transmit the effect using filtered supernatants were unsuccessful. However, transmission was demonstrated by X-irradiating affected cells with...
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Fig. 1.—Human embryonic fibroblasts following co-cultivation with human leukemic blood cells, showing giant cells with typical CMV inclusion bodies in nucleus and cytoplasm. Chromotrope 2R and Giemsa × 1000.

3000 rads and mixing 10⁴ of these with approximately 10⁶ human embryonic lung fibroblasts, showing that the entire culture became affected. Preparations stained with Chromotrope 2R Giemsa showed giant cells with large intranuclear and intracytoplasmic inclusion bodies (Fig. 1). Methyl green pyronin staining suggested that both nuclear and cytoplasmic inclusions contained DNA. When the infected cells were examined under an A.E.I. EM6B electron microscope, herpes-like particles were seen inside the nucleus in the areas thought to correspond to the nuclear inclusion bodies (Fig. 2). Direct examination of the blood in the electron microscope failed to reveal any virus particles.

The nature of the cytopathic effect, together with the morphology of the infected cells, the presence of herpes-like particles in the nucleus, and the
failure to demonstrate infective particles in the supernatant, suggest cytomegalovirus (CMV).\(^2,3\) A sample of infected cells was examined by Dr. Harold Stern of St. George’s Hospital, London, who confirmed that the agent was a cytomegalovirus by the following criteria: (1) the specific cytopathic effect in human fibroblasts, (2) the presence of intranuclear inclusion bodies, (3) the failure to subculture the virus to human epithelial cells, and (4) the demonstration that complement fixing antigen prepared from the infected cells reacted with a known positive human cytomegalovirus antiserum and failed to react with a known negative serum.

A second sample of blood was obtained from the patient and processed in exactly the same way as the first. Cytomegalovirus was again isolated after the same time interval as before.

A throat swab and a urine sample were obtained at the same time as the second blood sample. Both were heavily infected with yeast, but after passing the samples through a Millipore membrane (pore size 0.2\(\mu\), herpes simplex (identified by a specific neutralization test) was isolated from the oral swab and the culture was destroyed before any CMV which might have been

Fig. 2.—Human embryonic fibroblast following co-cultivation with human leukemic blood cells showing virus particles within the nuclear inclusion body. Glutaraldehyde osmic acid fixation, uranylacetate/lead citrate staining. \(\times 80,000\).
present could be demonstrated. No virus was detected in the urine and no inclusion bodies were seen in the cells sedimented from urine.

There was no clinical evidence of cytomegalic inclusion body disease (CID) and, since permission for postmortem was refused, there is no evidence except the isolation from peripheral blood that this child was infected with CMV.

We consider, however, that the double isolation indicates clearly that the virus did come from the patient. Since no free virus was detected, a specific neutralization test with the patient's own serum could not be carried out. Kaariainen et al.\textsuperscript{4} have shown an apparently high incidence of CMV infection in patients given massive fresh blood transfusions during open heart surgery. Although this is not a probable explanation of the presence of the virus, in this case since the last transfusion was given more than a year before the blood samples were taken, it seems possible that the method described might be useful in determining the proportion of donors who are carriers of CMV.

A similar experiment was set up with cells from a biopsy specimen of the neuroblastoma in the patient's sister, but no CMV was detected. One other experiment of the series was carried out using cells from an untreated child with acute lymphatic leukemia; again, no CMV was detected.

Evidence of infection with CMV has been reported in patients with leukemia on a number of occasions.\textsuperscript{5-7} Duvall et al.\textsuperscript{8} report an apparent specific association between CMV infection and neoplastic disease in adults. However, Benyesh-Melnick, Dessy, and Fernbach\textsuperscript{9} have shown that the frequency of CMV isolation in leukemic children (7/42) is no different from the incidence in children with other diseases (6/30) and in healthy children (6/29). There is further evidence that the virus may be isolated from a proportion of young healthy children,\textsuperscript{10,11} while 54 per cent of adults have acquired complement fixing antibodies against CMV by age 35.\textsuperscript{12}

The leukemic patient may be more likely to show clinical evidence of CID because of the debilitating nature of his leukemia\textsuperscript{5} or because of treatment with immunosuppressive drugs.\textsuperscript{6} The overt signs of CID in leukemia patients could be due to the activation of a latent infection or to a new infection. Bodey et al.\textsuperscript{6} are of the opinion that the cases they observed were new infections, since they occurred together in a cluster suggesting an increased prevalence of the virus at that time. The suggestion that the virus is more readily recovered from patients treated with immunosuppressive drugs is supported by the finding that cytomegalovirus can be detected in a high proportion of patients so treated during the course of renal homotransplantation procedures.\textsuperscript{13}

The weight of the present evidence does not suggest that CMV is an etiologic factor in human leukemia. However, we know of no other isolation of CMV from peripheral blood. The present report, therefore, may be of some interest, especially when human blood is being examined in the electron microscope for the presence of virus-like particles. Ames et al.\textsuperscript{14} recently reported finding virus-like particles in the urine of patients with leukemia and also the isolation of a virus, which was probably CMV, from these urines.
It is clearly important, therefore, to distinguish between type-C particles and CMV in electronmicrographs from human leukemic material.

A herpes-like virus has been observed in lines of cells established from tumor biopsies of patients with Burkitt's lymphoma\textsuperscript{15,16} and in cells of a similar line from a patient with chronic myeloid leukemia.\textsuperscript{17} It has, however, been demonstrated that these viruses are unlike any of the known herpes-viruses and are not cytomegalovirus.\textsuperscript{18}

The isolation of measles virus from the washed leukocyte fraction of peripheral blood has been reported,\textsuperscript{19-20} and Fenner and Woodroofe\textsuperscript{21} used this technic to demonstrate myxomatosis virus in rabbits. It seems possible that one might use this method to demonstrate CMV in suspected cases of CID or where it appears that CID may be a complicating factor in cases of leukemia or in other cases given large doses of immunosuppressive drugs.

**Summary**

Cytomegalovirus was isolated on two separate occasions from the washed leukocyte fraction of the peripheral blood of an 8-year-old boy with acute lymphatic leukemia. The child, who was treated, showed no other evidence of CMV infection. The relationship between CMV infection and leukemia is discussed.

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**ADDENDUM**

Since this manuscript was submitted for publication, Stulberg, Zeulzer, Page, Taylor and Brough (Proc. Soc. Exp. Biol. Med. 123:976, 1966) have reported the isolation of CMV from the peripheral blood of a month-old infant with hepatitis (giant cell type) using a technic very similar to the one described in this paper.

**REFERENCES**


Brief Report: The Isolation of Cytomegalovirus from Peripheral Blood