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Inability to Detect Hepatitis B Virus or Specific Antigens in Transformed Chimpanzee Lymphocytes

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Transformed chimpanzee lymphocytes were examined to determine whether they would support the replication of hepatitis B virus. After 5 months, no hepatitis B virus, hepatitis B surface antigen, or antibody to hepatitis B surface antigen was detected.

Although the viral etiology of hepatitis B has been firmly established and much progress has been made recently in the biophysical definition of the antigenic components of hepatitis B virus (HBV; 3, 4, 5, 6, 15), attempts to replicate this agent in a variety of in vitro cell culture systems have not to date been successful. Several recent studies have suggested that a surface antigenic component of HBV, now designated hepatitis B surface antigen (HB_s Ag), may have some effect on the transformation of lymphocytes in vitro (1, 9, 12, 13). Lymphocytes have been shown to support growth of several viral pathogens (11, 14). However, there have been no reports to date on the ability of transformed lymphocytes to support replication of HBV. Since the cause of hepatitis B virus infection of chimpanzees resembles that seen in humans (2, 10), including occurrence of the HB_s Ag chronic carrier state, we decided to examine transformed lymphocytes from this animal for possible evidence of HBV replication in vitro. If the chronic carrier state presupposes continuous replication of HBV, then lymphocytes from these carriers may provide a more suitable in vitro model for HBV replication.

Lymphocyte cultures from HB_s Ag-positive and normal chimpanzees were initiated. Heparinized whole blood was collected and mixed with Plasmagel (Roger Bellon Laboratories, Nevilly, France) to facilitate precipitation of erythrocytes. The leukocytes were removed, centrifuged, and mixed with a lysing buffer to destroy remaining erythrocytes. After centrifugation, the leukocytes were counted and planted at 5×10^6 cells per ml. No transforming agent was required since all our chimpanzees have Epstein-Barr virus antibody and Epstein-Barr virus is a known transforming agent (S. S. Kalter, R. L. Heberling, and J. J. Ratner, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1972, V45, p. 193).

Cultures from all sources transformed in 4 to 6 weeks. Growth medium was RPMI 1640 (Grand Island Biological Co., Grand Island, N.Y.) with 1% L-glutamine, 20% inactivated fetal calf serum and 1 mg of neomycin per ml. Maintenance medium was identical to growth medium except RPMI 1629 was used. The cultures were split 1:2 twice weekly. At biweekly intervals before and after transformation several methods were used for examining the lymphocytes over a period of 5 months. Also, transformed lymphocyte cultures from normal chimpanzees and chronic carriers were inoculated with a serum of known hepatitis B infectivity (MS-2; 8) and examined periodically.

Radioimmunoassay (Ausria-Abbott Laboratories) for HB_s Ag and anti-HB_s detection were performed on supernatant culture fluids and disrupted lymphocytes. The specimens were also examined for HB_s Ag and anti-HB_s after concentration by a polyacrylamide gel (Lyphogel, Gelman Co., Ann Arbor, Mich.) method. Passive hemagglutination (Electro-Nucleonics Laboratories, Inc., Bethesda, Md.), with microtiter equipment, was utilized for detection of anti-HB_s. The detection of hepatitis B core antigen (HB_c Ag) was attempted by a standard microtiter complement fixation method with core antibody obtained from J. Hoofnagle (7). Transformed lymphocytes were concentrated by centrifugation and then treated with Triton X-100 (Calbiochem, Los Angeles, Calif.). Indirect fluorescent antibody tests were done with a goat anti-guinea pig conjugate labeled with fluorescein isothiocyanate. For electron microscopy, the cells were fixed in 2% glutaraldehyde, dehydrated, stained with osmic acid, embedded in Epon, sectioned, stained with uranyl acetate and lead citrate, and examined in an RCA EMU-4A electron microscope.

Techniques described above for the detection of HB_s Ag, HB_c Ag, and anti-HB_s did not show

the production of antigen or antibody. By electron microscopy, Epstein-Barr virus was observed in the transformed lymphocytes but no particles resembling HB_s Ag or HB_c Ag were seen.

Since lymphocytes are an integral component of the immune system, it was felt that by closely following transformed chimpanzee lymphocytes from HB_s Ag and anti-HB_s carriers, the production of an HBV agent or a surface antigen might occur. We were unable to demonstrate the production of HB_s Ag or anti-HB_s or to visualize HBV or a surface antigen by electron microscopy. After inoculation of transformed lymphocytes with a known infective sample of MS-2, we were still unable to demonstrate positive results. Electron microscopy demonstrated the presence of Epstein-Barr virus in the transformed lymphocytes, and perhaps the inability to grow HBV in them may be because a double infection would not occur. Whether HBV replication in lymphocytes occurs in vivo in cells free of Epstein-Barr virus remains to be answered. It appears that transformed lymphocytes, whether from HB_s Ag-positive, anti-HB_s-positive, or normal chimpanzees, cannot support the growth of HBV or the production of antigens or antibodies to hepatitis B in vitro. This does not imply that all reticuloendothelial system cells could not support the growth of HBV. Further studies with chimpanzee macrophages and HBV are in progress.

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