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INDUCTION OF EBNA PRECEDES THE FIRST CELLULAR S-PHASE AFTER EBV-INFECTION OF HUMAN LYMPHOCYTES

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Using a combination of immunofluorescence and autoradiography, we studied the appearance of EBNA and DNA synthesis in cord-blood lymphocytes after infection with EBV derived from the B95-8 cell line. EBNA appeared between 12 and 25 h after addition of the virus. DNA synthesis was detected in EBNA-positive cells approximately 20 h after the appearance of EBNA. This shows that EBNA induction precedes the first cellular S-phase and suggests that the cells have not yet entered the cell-division cycle when EBNA appears. Little, if any, of the total DNA synthesis induced at this stage can be attributed to EBV-mediated immunologic stimulation.

blood cells was variable (5%-80%); only samples with less than 20% red blood cells were used for further experimentation. Subsequently cells were washed three times in serum-free medium. During the experiments the lymphocytes were incubated in RPMI 1640 medium with 100 IU/ml of penicillin, 200 μg/ml of streptomycin and 10% gamma-globulin-free calf serum.

Virus

EB-virus was produced by incubating the B95-8 cell line (Miller and Lipman, 1973) in RPMI medium with 10% fetal calf serum for 10 days. The cell super-