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INDUCTION OF EPSTEIN-BARR VIRUS ANTIGENS BY HYDROXYUREA

Z. NOVÁKOVÁ AND J. ROUBAL

Department of Experimental Virology, Institute of Sera and Vaccines, 101 03 Prague 10, Czechoslovakia

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Summary. — Treatment of the Epstein-Barr virus (EBV)- transformed, virus-producer P3HR-1 cell line with hydroxyurea (HU) resulted in increased synthesis of the EBV-specific early antigen (EA) and viral capsid antigen (VCA). The induction was noted already at a 200 μmol/l and reached plateau at a 1500 μmol/l HU concentration. At plateau concentration, the percentage of cells expressing EA and VCA was about 5 times higher than in the absence of the drug.

Key words: Epstein-Barr virus; virus antigen induction; hydroxyurea

Epstein-Barr virus (EBV) is a B-lymphotropic virus capable of immortalizing human B-cells both in vivo (Gerber *et al.*, 1969) and in vitro (Miller *et al.*, 1971). The transformed cells always contain EBV-DNA (Adams, 1979), express virus-specific latent membrane antigen (Moss *et al.*, 1981) and virus-encoded proteins of the nuclear antigen complex (EBNA complex) (Kallin *et al.*, 1986). In the so-called virus-nonproducer cell lines only these virus markers can be detected.

In some cell lines, known as virus producers, a small fraction of the cell population spontaneously enters into productive virus cycle. These events consecutively include the synthesis of virus-specific early antigen (EA), replication of viral DNA, synthesis of late membrane antigen, of viral capsid antigen (VCA) and formation of virus particles (Ernberg and Klein, 1979). A variety of substances and conditions can induce or enhance the synthesis of productive-virus-cycle antigens in virus nonproducer or virus producer cells, respectively. These substances and other factors include, e.g., halogenated pyrimidines (Hampar *et al.*, 1972; Gerber, 1972), azacytidine (Ben-Sasson and Klein, 1981), mitomycin-C (Moar and Klein, 1980), n-butyrate (Luka *et al.*, 1979), tumour promoters (zur Hausen *et al.*, 1978), cis-DDP (Vonka *et al.*, 1972a), arginine deprivation (Henle and Henle, 1968) and anti-IgM (Tovey *et al.*, 1978). In the present paper we report that also hydroxyurea (HU) can induce the synthesis of viral antigens in virus-producer P3HR-1 cells.

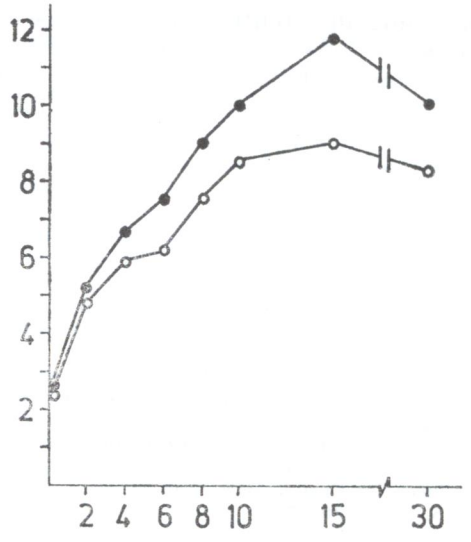

Fig. 1
Induction of virus antigens (EA and VCA) by hydroxyurea detected on days 3 (○——) and 5(●– –) after drug addition
Abscissa: Hydroxyurea concentration (μmol/l $\times$ 10^{-2})
Ordinate: Per cent of cells containing EA and VCA.

The Burkitt lymphoma cell line P3HR-1 (Hinuma and Grace, 1967) was cultivated as described previously (Vonka *et al.*, 1972b). It spontaneously expressed the antigens of the productive virus cycle in approximately 2.5 per cent of the cell population. For induction experiments, cells were grown to a high density, pelleted and resuspended in a fresh growth medium MEM (Sevac, Prague) to 5 $\times$ 10^5 cells/ml. Hydroxyurea was added to the final concentrations indicated in Fig. 1 and the cells were cultivated at 37 °C. On days 3 and 5 after addition of the drug, aliquots of cells were withdrawn for determination of EA- and VCA-content. The percentage of cells containing these antigens was determined on acetone-fixed smears by the indirect immunofluorescence technique (Henle and Henle, 1966) using serum from a patient with nasopharyngeal carcinoma and FITC-conjugated goat antihuman IgG (Hyland). EA and VCA were not distinguished.

Cell growth and cell viability was reduced with increasing HU concentrations. Counting the cells on day 3 after the addition of the drug showed 50 per cent growth inhibition at an 800 μmol/l HU concentration. Cell viability was somewhat less susceptible to the action of HU. The live cell count was reduced to 50 per cent at a 1600 μmol/l concentration while in untreated control cultures dead cells amounted to about 6 per cent on the average.

As shown in Fig. 1, the increase in viral antigen synthesis was detectable already on day 3 after addition of the drug. A further small increase was noted on day 5. Already 200 μmol/l HU was effective in inducing viral antigen synthesis: the percentage of EA- and VCA-producing cells was doubled. The inducing effect was more pronounced with increasing concentrations of the drug up to 1500 μmol/l, when the synthesis of EA plus VCA reached a plateau.

The mechanism by which HU can induce the synthesis of EBV antigens is not clear. The drug is known to inhibit semiconservative DNA replication (Krakoff *et al.*, 1968), thus blocking the synthesis of most of cellular DNA. On the other hand, the replication of viral DNA is insensitive to the action of the drug (Moar and Klein, 1980) owing to the drug resistance of virus-induced ribonucleoside reductase (Male *et al.*, 1974). It is probable that these properties of HU are involved in the mechanism of viral antigen induction: the disconcerting of the cellular and viral DNA replication

proceeding in latently infected cells can stimulate the entry of cells into productive EBV cycle. Indeed, interference with DNA synthesis is characteristic of various EBV-inducers (Gergely *et al.*, 1971) and is second in frequency only to the modulation of cell differentiation (Roubalová *et al.*, 1985; Anisimová *et al.*, 1984).

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