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EFFECT OF N-BUTYRATE ON SUPERINFECTION OF RAJI LYMPHOBLASTOID CELL LINE BY EPSTEIN-BARR VIRUS

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Summary. — The effect of n-butyrate on superinfectability of virus-nonproducer Raji cells by the P3HR-1 strain of Epstein-Barr virus (EBV) was investigated. n-Butyrate is known to be a potent inducer of virus antigen synthesis in virus-producer cell lines and of cell differentiation in virus nonproducers. The drug inhibited the growth of Raji cells but did not interfere markedly with cell viability. It induced a low rate of early antigen (EA) synthesis in about 1—2% of noninfected Raji cells. While the number of superinfectable cells remained relatively constant after treatment with butyrate, an increase in antigen positivity was noted in untreated cells. This relative decrease in sensitivity to superinfection in butyrate-treated Raji cells was more pronounced in cultures that had been treated with the drug for 48 or 72 hr as compared to those treated for 24 hr. A blocking of the treated cells in the certain cell-cycle phase and their drug-induced differentiation towards plasma cells might have been involved in the phenomenon described.

Key words: Epstein-Barr virus; lymphoblastoid cell lines; n-butyrate; superinfection

The Epstein-Barr virus (EBV) genome-carrying lymphoblastoid cell lines are usually divided into virus producers and virus nonproducers. In the former group a low proportion of cells can spontaneously enter on the productive virus cycle at any time; this is characterized by the synthesis of viral early antigen (EA), replication of viral DNA, synthesis of virus capsid antigen (VCA) and release of infectious virus (for review see Ernberg and Klein, 1979). In virus nonproducers, the virus genome expresses only an EBV-determined nuclear antigen (EBNA) (Reedman and Klein, 1973). In the cells of either group synthesis of other EBV antigens belonging to the productive virus cycle can be induced or enhanced by various chemical inducers (Ernberg and Moar, 1981; Gerber, 1972; Hampar *et al.*, 1974; Luka *et al.*, 1979; Magrath *et al.*, 1979; Tovey *et al.*, 1978; zur Hausen *et al.*, 1979). In addition, superinfection of nonproducer cells by EBV that has been

Table 1. Viability of Raji cells in the presence of 3 mmol/l n-butyrate

Hours after addition of n-butyrate	Per cent of dead cells	
	Butyrate-treated cells	Untreated cells
24	11.9	16.4
48	15.2	10.5
72	25.1	10.6

obtained from cultures of virus-producer P3HR-1 cells (Adams and Klein, 1973) also results in induction of the productive virus cycle.

n-Butyrate has been shown to be a potent inducer of virus-antigen synthesis (Luka *et al.*, 1979) and virus-particle formation (Anisimová, *et al.*, 1982) in the virus-producer P3HR-1 cell line. In virus nonproducers, it only induces the synthesis of EA in a few cells of the population. Interestingly, the drug induces morphological differentiation of some virus-nonproducer cells towards plasma cells (Anisimová *et al.*, 1982, 1984). The aim of the present study was to follow the effect of n-butyrate on superinfectability of Raji cells by the P3HR-1 strain of EBV.

The virus producer P3HR-1 cell line, derived from Burkitt lymphoma (BL) (Hinuma and Grace, 1967), served as a source of virus. The virus-nonproducer BL Raji cell line (Pulvertaft, 1965) was used for superinfection experiments. Both lines were cultivated in growth medium MEM as described previously (Vonka *et al.*, 1972). P3HR-1 virus was obtained by 100-fold concentration of the fluid phase of P3HR-1 cultures that had been aged 10 days at 33 °C.

The experiments were performed as follows: Raji cells were grown to a high density, centrifuged, and resuspended in fresh growth medium to 5×10^5 cells/ml. n-Butyrate was added to a 3 mmol/l final concentration. At three 24-hr intervals the medium containing n-butyrate was removed, the number of cells was adjusted to 1×10^6 viable cells/ml, and the cells were superinfected with P3HR-1 virus at a ratio of 1 ml of 1 : 2 diluted P3HR-1 virus stock per 10^6 viable cells. Virus adsorption lasted for 1 hr at 37 °C. Then the cells were spun down, adjusted to 5×10^5 viable cells/ml with fresh, butyrate-free growth medium and cultivated at 37 °C. Parallel cultures in which the addition of either n-butyrate or virus was omitted served as controls. On the third day after superinfection, acetone-fixed smears were prepared for detection of cells containing viral antigens. The detection was done by the indirect immunofluorescence (IF) technique of Henle and Henle (1966) using human sera with a high titre of antibodies against both EA and VCA. However, EA and VCA were not distinquished. At least 500 cells were counted to determine the relative number of virus antigen-positive cells.

During 48-hr incubation n-butyrate inhibited the growth of Raji cells but did not significantly influence their viability (Table 1).

As shown in Table 2, the number of antigen-positive Raji cells remained relatively constant in superinfected butyrate-treated cultures, while it increased in superinfected untreated controls. This indicates that treated cells were relatively less sensitive to superinfection by the P3HR-1 strain of EBV. The relative reduction in the number of cells positive in the IF test was greater if the Raji cells had been treated with n-butyrate for 48 or 72 hr than for 24 hr. A low rate induction of EA (1—2% of EA-positive

Table 2. Per cent of EA-positive Raji cells pretreated with n-butyrate and superinfected with P3HR-1 virus[1]

Superinfection: hr after addition of n-butyrate[2])	Per cent of EA-positive cells		Relative reduction in number of EA-positive cells in pretreated cultures (%)
	Untreated	Pretreated	
24	10.9	8.70	20.8
48	15.3	9.17	40.1
72	17.2	9.55	44.6

[1]) No EA-positive cells were found in untreated, nonsuperinfected cells
[2]) Nonsuperinfected, butyrate-treated cells exhibited a low rate of EA induction: 1—2% of the cell population were positive in IF test

cells) was observed in nonsuperinfected Raji cells that had been treated with n-butyrate for 72 hr. No cells positive for virus antigens were found in untreated Raji cultures.

The differences in superinfectability between butyrate-treated and control Raji cells could not be explained by competition of the receptors at the surface of dead cells for inoculated virus. The differences in the numbers of dead cells between the cultures were not high enough to allow such a conclusion.

A blocking by butyrate of the cell cycle (Daniell, 1980) and recently described Raji cell differentiation in the direction of plasma cells (Anisimová *et al.*, 1982) may have been involved in the phenomenon observed, since plasma cells do not possess receptors for EBV (Nilsson, 1978) and since the receptor concentration depends on the cell cycle (Wells *et al.*, 1981). The loss of receptors during butyrate-driven plasma-cell maturation and the blocking of their reappearance in dependence on cell cycle may have been responsible for the relatively constant number of superinfectable Raji cells in n-butyrate--treated cultures (Table 2). The lack of these influences in control cultures would then have resulted in their stepwise increase in sensitivity to superinfection. Alternatively, drug-induced changes in the intracellular condition might have been in the background of the effect described.

The suggestions seem to be in line with findings of other authors. Yamamoto *et al.* (1982) have reported a 50% decrease in concentration of EBV receptors in Raji cells that have been treated with 12-0-tetradecanoylphorbol--13-acetate. Interestingly enough, this drug can also induce differentiation-related changes in Raji cells (Anisimová *et al.*, 1984). In addition, Einhorn and Klein (1981) have recently demonstrated that protein A-induced differentiation of B-lymphocytes results in a decreased frequency of their transformation by the B95-8 strain of EBV. This, as well as the differences in transformability by EBV of cells from various B-cell lymphomas or leukaemias that differ by the stage of B-cell differentiation (Finerty *et al.*, 1982; Rickinson *et al.*, 1982), may be attributed either to a variance in EBV receptor expression or differences in intracellular conditions.

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