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Superinfection of EBV-Carrying Lines with Herpes Virus Papio (HVP): Induction of Early Antigen (EA) and Inhibition of Cellular DNA Synthesis*

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Abstract—*An Epstein–Barr virus (EBV) related herpes virus H. papio (HVP) derived from 9B cell line was shown to inhibit the DNA synthesis in B-cell mitogen stimulated human lymphocytes, in B95-8 virus exposed human lymphocytes, and the DNA metabolism of EBV-receptor positive cell lines. The inhibition could be abolished by a Burkitt serum known to have a high anti-EBV titer. Similar inhibition activities were shown for P3HR-1 virus. The relationship between the inability to immortalize normal cells, the ability to induce early antigen (EA) in certain cell lines and the DNA inhibitory effect of both 9B and P3HR-1 viruses is discussed.*

INTRODUCTION

TWO LABORATORY substrains of the Epstein–Barr virus (EBV), derived from the B95-8 [1] and the P3HR-1 lines [2] have been studied most extensively. They show considerable difference in their biological properties. B95-8 virus induces the nuclear antigen EBNA and stimulates DNA synthesis in normal B lymphocytes of human or some non-human primate origin. This is followed by transformation (immortalization) into permanent lines [1, 3]. In contrast, P3HR-1 does not transform, and fails to induce EBNA and cellular DNA synthesis [3]. There is less of a difference in the action of the two viruses on the EBV negative but EBV-susceptible BJAB and Ramos lines [4, 5]. They could be converted into permanent EBV-carriers by both virus substrains [6, 7]. Nevertheless, there are considerable differences between the two virus strains even on established B-cell lines. EBV-receptor carrying lines are inhibited by P3HR-1 but not by B95-8 virus [8]. The P3HR-1 virus appears to inhibit the cellular DNA synthesis in them. A similar inhibitory action on cellular DNA synthesis was found when P3HR-1 virus was added to normal B-lymphocytes before, simultaneously or immediately after the addition of the stimulatory B95-8 virus or the B-cell mitogen *Staphylococcus aureus* [9].

The P3HR-1 virus was also found to be unique in one other respect among human EBV-isolates tested. Unlike transforming EBV, P3HR-1 virus induces early antigen (EA) in Raji cells and other EBV receptor positive cell lines [10].

Another way to induce EA in non-producer lines is treatment with the halogenated pyrimidines, IUDR and BUDR [11]. Like P3HR-1 virus, both chemicals interfere with the host cell DNA synthesis. It is conceivable that the EA inducing effect of the P3HR-1 virus is also related to its inhibitory effect on host cell DNA synthesis, particularly since it was recently shown that the resident EBV-genome participates in EA induction by P3HR-1 viral superinfection [12].

Recently an EBV related baboon herpesvirus H. Papio (HVP) was discovered [13]. HVP is a relative of EBV, with approximately 10% DNA homology. Moreover, the viral antigens EA and VCA cross react with the corresponding EBV-determined antigen [14]. HVP derived from the baboon virus producer lymphoid line 9B could induce EA and VCA in Raji cells and EA in BJAB and 26 CB-1

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cell lines. Like EBV transformed lines, HVP-carrying lines carry multiple copies of the viral genome. One difference is that HVP-carrying lines do not express an *in situ* stainable nuclear antigen like EBNA, but they have an analogous nuclear antigen, HUPNA, which can be demonstrated by the amplification provided by the acid fixed nuclear binding (AFNB) technique [15]. HUPNA partially cross reacts with its human counterpart, EBNA [16].

The biological properties of HVP are not well known. Transformation (immortalization) of normal lymphocytes was not achieved by infection with the cell free virus, but could be obtained after cocultivation with irradiated donor cells [17].

The present paper deals with the question whether EA-inducing ability of the 9B virus is correlated with an inhibition of DNA synthesis, as in the case of P3HR-1 variant of EBV.

MATERIALS AND METHODS

Lymphocytes

The Ficoll–Isopaque method of Böyum [18] was used to isolate lymphocytes from the peripheral blood of normal human donors.

Thymidine incorporation test

Lymphocytes were suspended in RPMI-1640 supplemented with 10% Ig free fetal calf serum (Gibco) in a final concentration of 10^7 cells/ml. Cells derived from cultured lines were suspended in RPMI-1640 supplemented with 10% fetal calf serum in a final concentration of 0.5×10^4cells/ml. To test the effect of P3HR-1 and 9B derived virus of DNA synthesis, 0.02 ml cell suspension aliquots were exposed to 0.02 ml of either one of the two virus preparations in Falcon 3040 microplates. After 2 hr incubation at 37°C in 5% CO_2, 0.02 ml B95-8 virus was added for 120 min or, alternatively, 0.15 ml medium containing *Staphylococcus aureus* on PHA, 1 μCi ^{3}H thymidine was added for the final 24 hr of incubation. Subsequently, the cells were harvested on filter papers and the incorporated counts were assessed in a scintillation counter. Four to five replicates were performed for each test: they differed by less than 20% as a rule. Inhibition of thymidine incorporation was calculated according to the following formula:

$$\% \text{ inhibition} = 100 - \frac{A-B}{C-B} \times 100.$$

A—CPM of 9B or P3HR-1 virus exposed cells, subsequently exposed to mitogen or to B95-8 virus.

B—Background CPM of untreated cells.

C—CPM of the cells exposed to mitogen or to B95-8 virus.

Viruses: HVP-9B virus was concentrated [19] from the supernatant of 9B cell line [13]. One milliliter 1:16 diluted virus induced 13% early antigen (EA) positive cells 48 hr after the infection of 10^6 Raji cells [14].

P3HR-1 virus was concentrated in the same way as 9B virus. 1:2.5 dilution was used. One milliliter 1:10 diluted virus induced early antigen (EA) in 11% of 10^6 Raji cells after 48 hr.

B95-8 virus: The culture supernatant of the B95-8 cell line [1] was used without further concentration. It induced 20–28% EBNA positive nuclei 48 hr after the infection of 10^6 EBV negative Ramos cells.

Staphylococcus aureus: formalin fixed *Staphylococcus aureus* [20] was used as a human B cell mitogen [9]. The final concentrations corresponded to a 50 and 25 fild excess over the number of the lymphocytes.

PHA: PHA (leucoagglutinin, Pharmacia) was added in a final concentration of 1 μg/ml.

RESULTS

Table 1 shows the effect of 9B and P3HR-1 virus pretreatment on thymidine incorporation into B95-8 virus, *Staph. aureus* and PHA stimulated lymphocytes. Both virus populations inhibited the DNA stimulating effect of B95-8 virus and of the B cell mitogen, *Staph. aureus*. In contrast, the T cell mitogen (PHA) induced DNA stimulation was not inhibited.

Table 2 shows that 9B virus inhibited the DNA synthesis in EBV receptor positive lines to 45–80%. Pretreatment of the virus with an EBV-neutralizing Burkitt lymphoma serum abolished the DNA synthesis inhibiting activity of the virus. P3HR-1 inhibited DNA metabolism in EBV receptor positive cells, also. Pretreatment with the same Burkitt serum abolished the effect of the virus.

The inhibiting effect was not due to residual viral genome as indicated by considerable inhibition manifested in both Ramos and BJAB cell lines. However, K562, lacking EBV receptor was not inhibited. In additional experiments not shown here, the viruses did not inhibit Rael cell line (EBNA positive, EBV receptor negative) and Molt-4 line (EBNA negative, EBV receptor positive). Molt-4 which has EBV receptor but no EBNA cannot

Table 1. *Effect of P3HR-1 or 9B derived virus pretreatment on the DNA stimulating effect of* Staphylococcus aureus, B95-8 *virus and PHA*

First exposure (virus source and dilution)	Control medium (CPM)	Second exposure *Staph. aureus* 50/1* (CPM)	*Staph. aureus* 25/1 (CPM)	B95-8 virus (CPM)	PHA (CPM)
—	780	10642	11922	13365	29742
	(CPM)		%-Inhibition		
9B (1:5)	732	65	71	98	0
9B (1:50)	1477	14	15	83	14
P3HR-1 (1:5)	701	60	68	95	12

*Ratio of *Staphylococcus aureus* particles/lymphocyte.

Human peripheral lymphocytes were exposed for 2 hr to 9B or P3HR-1 virus. Subsequently, they were exposed to *Staph. aureus*, B95-8 virus or PHA. The experiment was terminated after 3 days (for *Staph. aureus* and PHA stimulation tests) or 7 days (for B95-8 stimulation test). 1 μCi ^{3}H thymidine was added for the final 24 hr of incubation.

be induced by superinfection with EBV. It appears that inhibition of DNA is manifested only in cells with a functionally active EBV receptor, as indicated also in the susceptibility of the cell to EBNA induction by EBV superinfection. No inhibition was caused by infection with B95-8 virus.

DISCUSSION

Among all the known human EBV-isolates, P3HR-1 virus has been unique so far in the following properties: (i) It fails to transform normal B-lymphoctes; (ii) it inhibits the DNA synthesis of EBV receptor positive established lines; (iii) it prevents the DNA synthesis stimulating effect of other transforming (B95-8) virus and of B-cell mitogens; (iv) it induces EA in Raji and other EBV-carrying lines.

HVP is the EBV related, lymphotropic herpesvirus of the baboon [13]. It shows many similarities to the EBV system, with partially homologous DNA sequences and cross reactive viral (EA and VCA) antigens [13, 14]. A notable difference between HVP and EBV is the absence of an *in situ* stainable nuclear antigen in the former, although an EBNA-like, cross reactive nuclear antigen can be demonstrated by the more sensitive AFNB amplification technique.

In a previous paper [14] we have shown that HVP-derived from the 9B and 18C strains induce EA in the Raji line. In this respect, these HVP isolates resembled, with regard to biological properties, the unusual P3HR-1 isolate rather than the more usual B95-8 transforming virus.

The unique properties of the P3HR-1 virus listed above may be either coincidental or directly related to each other. In particular, the inability of the virus to transform and its ability to induce the EA in Raji and other susceptible cells may be related to the DNA synthesis inhibitory effect of this virus. A relationship is suggested by the fact that DNA synthesis is a necessary prerequisite for transformation [24]. As for the EA induction in EBV carrying non-producer lines, it has been earlier shown [12] that the resident genome participates in the process. Induction can also be achieved, in the same cell lines, by incorporating IUDR into the genome of the target cell lines. This agent also interferes with DNA synthesis. A good correlation between IUDR and P3HR-1 viral superinfection, in inducing EA synthesis suggested that the common denominator may be the interference with the host cell DNA synthesis.

The availability of an independent isolate, derived from a closely similar but nevertheless distinct virus, HVP allowed to check some aspects of this hypothesis. In the present paper, we show that the 9B virus has the same DNA synthesis inhibitory properties, both in established, EBV-receptor carrying lines and in normal lymphocytes stimulated with transforming (B95-8) virus or with the B-cell mitogens, as P3HR-1 virus. It is therefore most likely that interference with the DNA syn-

Table 2. Inhibition of thymidine incorporation (%)

Cell line*	EBV receptor	EBNA	Native 9B-virus (dilution)		Neutralized 9B-virus (dilution)		Native P3HR-1 virus (dilution)			Neutralized P3HR-1 virus	Neutralizing serum only	Native B95-8 virus
			1:3	1:5	1:3	1:5	1:3	1:5	1:10	1:5		1:2
Raji	+	+	75	63	24	18	33	17	17	0	0	1
Daudi	+	+	70	57	9	2	N.D.†	85	N.D.	N.D.	N.D.	8
BJAB	+	−	47	45	7	0	33	13	21	0	0	0
BJAB/HR1K	+	+	76	69	36	8	47	13	0	0	0	0
BJAB/B95-8	+	+	72	69	12	0	46	27	9	0	0	N.D.
Ramos	+	−	58	59	5	0	37	20	10	0	8	12
EHRA Ramos	+	+	57	62	0	0	54	32	13	13	7	0
H WA Ramos	+	+	79	80	44	24	22	0	0	0	0	N.D.
K562	−	−	8	N.D.	0	0	0	0	3	0	0	0
Molt-4	−	−	1	N.D.	10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0

Effect of 9B, P3HR-1 and B95-8 viruses on the DNA synthesis in cell lines.
10^4 cells suspended in 0.02 ml medium were exposed to 9B virus or P3HR-1 virus or B95-8 virus for 2 hr at 37° C. Neutralized virus was tested in parallel. Neutralization was performed by pretreatment of the virus preparation in 1/10 diluted Burkitt lymphoma patient serum for 60 min. The serum used was Mutua previously known to have high EBV neutralizing activity [23].
*For references for the cell lines see [21] and [22].
†N.D. = Not done.

thesis of the target cell line played an important role in EA induction and also, in all probability, with regard to the other biological characteristics of the P3HR-1 virus, listed in the beginning of this discussion. In this context, it is noteworthy that a cell free HVP has so far not been able to transform normal lymphocytes into established lines [17].

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