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SHORT COMMUNICATION

INDUCTION OF NUCLEAR DEOXYRIBOPYRIMIDINE TRIPHOSPHATASE AND SENSITIVITY OF CLINICAL ISOLATES OF HERPES SIMPLEX VIRUS TO (*E*)-5-(2-BROMOVINYL)-2′-DEOXYURIDINE

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In a blind study, 21 clinical isolates of herpes simplex virus which had been typed using differential growth on guinea pig embryo versus chicken embryo cells were tested for the presence of the viral deoxyribopyrimidine triphosphatase. In all isolates of type 1, the triphosphatase was present in the nuclei of the infected cells, while none of the HSV-2 isolates induced a nuclear enzyme. In all isolates there was a complete correlation between the presence of nuclear deoxyribopyrimidine triphosphatase and sensitivity to 0.7 μg/ml of *E*-5-(2-bromovinyl)-2′-deoxyuridine. The study suggests that the type-specific distribution of the triphosphatase is of general validity, including clinical isolates of herpes simplex virus, and could be used as a type-specific enzyme marker.

herpes simplex virus; type specificity; BrvdUdr; dPyTPase

Since the recognition of two distinct serotypes of herpes simplex virus, the oral type 1 and genital type 2 (HSV-1 and HSV-2), a variety of characteristics has been discovered which distinguishes the two despite their close genetic and serological relationship. Differences are demonstrable by plaque morphology, serology, virion and infected cell polypeptides, host range, neurovirulence, and DNA restriction endonuclease analysis [7,9,10, 12].

While some of the biological and pathogenic properties of the two types differ considerably, the known virus-specific enzymes of DNA replication and nucleotide metabolism are rather similar. However, a number of potent antiviral and nucleotide analogues display differential activity against type 1 and type 2 [4]. For instance, the antiviral deoxythymidine analogue *E*-5-(2-bromovinyl)-2′-deoxyuridine (BrvdUrd) is 100–200-fold more active against HSV-1 than against HSV-2 [4]. It has been shown that the

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triphosphate of the drug can be incorporated into both HSV-1 and HSV-2 by the viral DNA polymerase [2].

We have recently demonstrated that the herpes simplex induced deoxyribopyrimidine triphosphatase (dPyTPase) shows type specificity with respect to its distribution within the infected cell [16]. The results showed that after disruption of the cells infected with two laboratory strains of HSV-1 (strains 17 and KOS), the dPyTPase is predominantly nuclear, whereas the enzyme is found exclusively in the cytoplasmic extract of cells infected with two laboratory strains of HSV-2 (strains HG52 and 186). The dPyTPase catalyzes the production of deoxyribopyrimidine monophosphate and inorganic pyrophosphate from the corresponding triphosphate in a reaction which proceeds in vitro at 4°C [15]. A similar activity has been described by Caradonna and Cheng [3]. Although the in vivo function of the enzyme is unknown, several findings, such as the low K_m values for the substrates, the distribution in the infected cell, and the substrate specificity suggest that the dPyTPase may play a key role in type specific pyrimidine deoxyribose metabolism.

Because of the potential clinical significance of these results, the present study analyzes dPyTPase levels in a number of clinical isolates, with the following aims: (1) to correlate the expression of the dPyTPase with the HSV-serotype, and (2) to test whether the dPyTPase can be used as a type-specific marker enzyme.

The blind study presented in Table 1 was performed in the following way: the isolates were obtained in the VA Hospital, West Haven, and typed by two methods, differential growth on chicken embryo versus guinea pig embryo cells [13], and susceptibility to BrvdUrd inhibition [4,5,11]. The isolates were then coded and sent to Yale University, where they were analyzed for expression of nuclear deoxyribopyrimidine triphosphatase. The nuclear enzyme levels were determined [15], since in contrast to the cytoplasm, this fraction gave a clear-cut diagnostic difference for laboratory strains of herpes simplex virus [16]. As shown in Table 1, all strains judged to be type 1 by host range and BrvdUrd sensitivity expressed nuclear dPyTPase, whereas all type 2 viruses were negative for the nuclear enzyme. It therefore appears that induction of dPyTPase in nuclei of infected BHK-cells is a general property of HSV-1.

The BrvdUrd typing method was originally proposed by De Clercq et al. [4] and is being used by us routinely to type clinical HSV-isolates [11]. The correlation between sensitivity to inhibition by BrvdUrd and nuclear dPyTPase expression in this study is striking, but most likely coincidental. The reason for the type-specificity of BrvdUrd action seems to be connected to the viral deoxyribopyrimidine kinase as judged by physical mapping data using intertypic recombinants (F. Wohlrab and B. Francke, unpublished results) and analysis of nucleotide pools in infected cells exposed to an analogous drug [6] and specificity studies with the virus-coded dThd-dTMP kinase [8]. However, the fact that both the kinase and the triphosphatase exhibit type-specific characteristics suggests that a fundamental difference in pyrimidine deoxyribose metabolism exists in HSV-1 and HSV-2 infected cells, and should be of considerable interest for the design and the evaluation of new and existing anti-herpes agents.

TABLE 1

Type specific expression of deoxypyrimidine triphosphatase induced by clinical isolates of HSV

Strain[a]	Virus titer (log_{10} p.f.u./0.1 ml)			Type[d]	Nuclear dPyTPase[e]	
	CE[b]	GPE[b]	GPE+BrvdUrd[c]		Activity	Rating
1867	4.5	4.6	4.7	2	13	-
1877	< 1	5.4	2.8	1	416	+
1897	< 1	5.8	3.8	1	736	+
1935	< 1	5.9	3.2	1	663	+
1943	3.1	3.0	3.4	2	31	-
1945	6.5	6.3	6.2	2	23	-
1960	< 1	6.2	2.8	1	547	+
1967	< 1	5.5	2.0	1	560	+
1968	< 1	5.3	2.2	1	1036	+
1978	< 1	6.0	3.8	1	992	+
2015	4.3	4.6	4.4	2	21	-
2016	4.3	4.4	4.3	2	28	-
2018	< 1	4.8	2.1	1	226	+
2020	2.2	4.8	2.7	1	815	+
2040	4.1	4.2	4.2	2	26	-
2041	5.2	5.7	5.6	2	24	-
2054	< 1	6.5	2.9	1	1050	+
2059	< 1	7.3	4.0	1	945	+
2073	3.8	4.4	4.4	2	24	-
2103	< 1	5.4	3.1	1	793	+
2113	< 1	4.9	1.6	1	745	+

[a] Virus isolates were characterized by plating on primary guinea pig embryo and chicken embryo monolayers in Linbro microtest plates as described [1, 13].

[b] Virus titers on chicken embryo (CE) and guinea pig embryo (GPE) cells.

[c] Susceptibility to BrvdUrd was determined by plating of virus isolates on guinea pig embryo monolayers in presence of 0.7 μg/ml BrvdUrd in the overlay.

[d] Virus type as determined by differential growth on CE and GPE cells.

[e] Nuclear dPyTPase was determined in isolated nuclei as described [15]. Baby hamster kidney cells were infected with each isolate at a multiplicity of infection of 0.01 and harvested 48 h after infection at 31.5°C. In parallel experiments, isolates were grown to high titer stocks and then used to infect cells at a multiplicity of 1 for 18 h at 31.5°C. The results were in each case comparable. After harvesting, cells were disrupted hypotonically and the nuclei isolated by centrifugation. The dPyTPase assay mixture contained in a final volume of 50 μl: 1 mM [^{3}H]dUTP (0.1 Ci/mmol) (Amersham), 1 mM dithiothreitol, 1 mM ethylene glycol bis-(β-aminoethylether)-*N,N,N',N'*-tetra-acetic acid (EGTA), 2 mM ATP, 10 mM $MgCl_2$, and 3×10^6 nuclei. Reactions were performed at 4°C for 15 min and terminated by adding 20 μl 0.1 M EDTA and 112.5 μl cold methanol. After separation of deoxynucleotides on polyethyleneimine-cellulose strips, the radioactivity of the reaction products was determined by liquid scintillation counting as described [12]. Activity is expressed as pmol dUMP formed/min per 10^7 nuclei. Isolates inducing significant amounts of dPyTPase in nuclei of infected cells (> 100 pmol/min per 10^7 nuclei, 4 times the highest activity found in nuclei from uninfected cells) were rated as +, those expressing less activity than 100 pmol/min per 10^7 nuclei were rated as -.

The complete correlation of virus-type with expression of nuclear dPyTPase indicates that the enzyme can be used as a type-specific marker. It is of interest that the mapping data presented elsewhere [16] define the locus governing the type-specificity of dPyTPase at 0.67–0.68 fractional genome length in a region of the HSV genome which has been shown to contain genes which are not colinear in HSV-1 and HSV-2 (such as glycoprotein C) [14]. It is conceivable that the difference in deoxyribopyrimidine nucleotide metabolism between the two serotypes of HSV might be partly a consequence of the compartmentalization of the enzymes involved.

In any case, the existence of a type-specific dPyTPase activity in herpes simplex infected cells suggests the necessity to examine this enzyme in studies of sensitivity and resistance to pyrimidine deoxyribose-based drugs.

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