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NOTES

Serological Study of a Mutant of Herpesvirus Unable to Stimulate Thymidine Kinase

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A mutant of herpes simplex virus which was unable to produce thymidine kinase also failed to produce an antigen which blocked the enzyme-inhibiting activity of antiserum prepared against virus-infected cells.

It has been suggested that herpesvirus deoxyribonucleic acid (DNA) codes for a new thymidine kinase (EC 2.7.1.21), since antiserum prepared against herpesvirus-infected RK13 cells inhibits the enzyme activity in virus-infected BHK-21 cells (5) and a mutant of herpesvirus was unable to stimulate thymidine kinase (2). These observations could be explained, however, by the failure of the mutant to produce an inducer of a host thymidine kinase to which a normal virus-coded antigen attached; i.e., antibody could then possibly react with the virus antigen moiety in such a way as to inhibit the enzyme activity. We have studied this alternative hypothesis by testing extracts of cells infected with the mutant virus for "serum-blocking" activity.

The parent wild-type and mutant (B2006; 2) herpesviruses were grown as described by Holmes and Watson (3). The preparation of cell extracts, assay of thymidine kinase, and production of antiserum have been described previously (5, 6). In serum-blocking tests, equal volumes (0.2 ml) of dilutions of infected or uninfected cell extracts were incubated overnight at 4 C with preimmune serum or antiserum. The mixture was centrifuged at 2,000 $\times$ *g* for 15 min and heated at 56 C for 30 min to inactivate thymidine kinase activity. This treatment does not affect the enzyme-neutralizing activity of the antiserum. The degree of blocking was measured by incubating 0.1 ml of the blocked serum with 0.1 ml of a known activity of herpesvirus thymidine kinase. After overnight incubation at 4 C, the mixture was centrifuged as above and the residual enzyme was measured.

It was found that although the virus-stimulated enzyme was inactivated at 56 C for 30 min in the presence of preimmune serum, immune serum protected a fraction of the enzyme activity from heat inactivation if the enzyme was present in excess. The protected kinase activity was determined as follows. A 0.2-ml amount of serum was blocked with infected cell extract as above. After overnight incubation, the mixture was heated at 56 C for 30 min and 0.1 ml was further incubated overnight at 4 C with 0.1 ml of infected cell extract previously heated at 56 C for 30 min to abolish all enzyme activity. The residual enzyme activity in the blocked serum was assayed.

Neither uninfected cell extracts nor extracts of cells infected with the mutant virus demonstrated blocking activity (Table 1). Concentrated extracts of uninfected cells and cells infected with the mutant virus also failed to show blocking activity (data not shown). Extracts of cells infected with the parent wild-type virus showed various degrees of blocking, depending on the amount of extract present.

These results show that the mutant virus fails to produce a protein which could attach to an induced host thymidine kinase. This lack of serum-blocking power, together with the data of Dubbs and Kit (2), Kit, Dubbs, and Anken (4), Klemperer et al. (5), and Buchan and Watson (1), suggests that the DNA of herpesvirus codes for a new thymidine kinase.

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TABLE 1. *"Serum blocking" activity of herpesvirus-infected cell extracts*

Blocked serum	Blocking extract	Residual thymidine kinase activity of blocked serum[a]	Enzyme activity after reaction with blocked serum	Enzyme activity after reaction with blocked serum corrected for residual activity[a]	
		counts per min per assay	*counts per min per assay*	*counts per min per assay*	%
Preimmune serum	Uninfected cell extract	0	9,290	9,290	100
Antiserum[b]	Uninfected cell extract	0	1,000	1,000	11
Preimmune serum	Herpesvirus-infected cell extract	0	8,665	8,665	93
Antiserum	Herpesvirus-infected cell extract	9,075	17,275	8,200	88
Antiserum	Herpesvirus-infected cell extract diluted 1:5[c]	565	6,900	6,425	69
Antiserum	Herpesvirus-infected cell extract diluted 1:10	0	1,865	1,865	20
Preimmune serum	Mutant virus-infected cell extract	0	10,082	10,082	110
Antiserum	Mutant virus-infected cell extract	0	0	0	0

[a] Cell extracts were tested for serum blocking power by incubation with serum overnight at 4 C. Residual kinase activity which was stable to heating at 56 C for 30 min was determined prior to addition of a previously assayed sample of thymidine kinase to determine the neutralizing activity of the blocked serum.

[b] Antiserum was prepared in rabbits by inoculation of rabbit kidney cells infected with herpesvirus.

[c] Extracts were diluted in 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride buffer (*p*H 7.5) containing 0.05 M NaCl.

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