The Immunological Specificity of Thymidine Kinases in Cells Infected by Viruses of the Herpes Group

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Two viruses of the herpes group, herpes simplex virus and pseudorabies virus, cause an increase in the activity of thymidine kinase (E.C. 2.7.1.20) in baby hamster kidney cells (BHK 21) and primary rabbit kidney cells respectively (Klemperer et al. 1967; Hamada, Kamiya & Kaplan, 1966). Klemperer et al. (1967) showed that the thymidine kinase produced in cells infected with herpes simplex virus was inhibited by antiserum prepared against RK 13 cells (Beale, Christofinis & Furminger, 1963) infected with the same virus. Similar results were obtained by Hamada et al. (1966) using thymidine kinase induced by pseudorabies virus and antiserum prepared against cells infected with pseudorabies virus.

Hamada et al. (1966) suggested that the increased activity and altered serological properties of thymidine kinase might have been the result of allosteric alterations in the

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Fig. 1. Inhibition by antiserum of thymidine kinase induced by herpes virus. Enzyme activity (in \( \mu \)mole of thymidine phosphorylated per 0.25 ml. reaction mixture) was measured at pH 6. Varying dilutions of infected cell extract were mixed with an equal volume of serum to give the ratios 2:1, 6:1, 18:1. Enzyme activity was determined after overnight incubation at 4°C. Enzyme + pre-immunization serum (\( \bigcirc-\bigcirc \)); enzyme + antiserum to herpes virus-infected RK cells (\( \Delta-\Delta \)); enzyme + antiserum to pseudorabies virus-infected RK cells (\( \square-\square \)).
enzyme caused by environmental changes within the infected cell. We compare, in this communication, the immunological properties of the thymidine kinases induced by herpes virus and pseudorabies virus in an attempt to shed some light on the nature of the genetic material (viral or host) specifying the enzymes.

Herpes simplex virus (HFEM strain) and pseudorabies virus (DEKKING strain) were grown in BHK 21 cells (Macpherson & Stoker, 1962) or RK 13 cells (Beale et al. 1963) by the methods described by Watson et al. (1966). Cell extracts were prepared and thymidine kinase activity determined as described by Klemperer et al. (1967). Briefly, infected cells were disrupted by sonication and a supernatant obtained by centrifuging at 100,000 g for 30 min. in a Christ Omega II ultracentrifuge. Aliquots of the supernatant fluid (20 to 100 µg. protein) were incubated in reaction mixtures containing, in a final volume of 0.25 ml., 10⁻⁶ M-thymidine-2⁻¹⁴C (0.1 µC), 5 mM-ATP, 5 mM-MgCl₂, and 0.02 M-sodium phosphate buffer, pH 6. Tubes were incubated for 10 min. at 37°, heated for 2 min. at 100°, then cooled in ice. The denatured protein was removed by centrifuging at 2000 g for 10 min. and 0.05 ml. of the supernatant fluid was chromatographed on DEAE cellulose paper. The region of the DEAE paper containing radioactive thymidylic acid was cut out and counted in a liquid scintillation counter.

Antisera were produced in rabbits by the inoculation of virus-infected RK 13 cells (Watson et al. 1966). Enzyme inhibition studies were made by mixing equal volumes of serum and dilutions of enzyme. After standing overnight at 4° the samples were diluted to the same supernatant protein concentration and assayed.

Fig. 2. Inhibition by antiserum of thymidine kinase induced by pseudorabies virus. Details are similar to those in Fig. 1.
The thymidine kinase activity induced by herpes virus was inhibited slightly at high pre-immunization serum concentrations and was unaffected by the antiserum against pseudorabies virus-infected cells (Fig. 1). The enzyme was, however, readily inhibited by the antiserum prepared against cells infected with herpes virus.

The same sera were tested on thymidine kinase induced by pseudorabies virus. The enzyme was not significantly affected by either the pre-immunization or the heterologous herpes virus antiserum. The homologous serum against cells infected with pseudorabies virus inhibited the enzyme activity by 90% at high serum concentrations (Fig. 2).

These results indicate that the thymidine kinases produced in the same cell type by two different viruses are serologically distinct and hence probably have different primary structures (Levine, 1962). It is unlikely that the immunologically specific differences between both enzymes could be caused by a non-specific environmental alteration resulting from infection as was suggested by Hamada et al. (1966). The most obvious interpretation of the results, therefore, is that the genetic material of each virus specifies a new thymidine kinase. However, the possibilities that either a virus-specified protein attaches to the host enzyme, thereby altering its biochemical and serological properties, or the new activities arise by de-repression of host-cell thymidine kinases have not been eliminated. The latter is probably unlikely.

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Department of Virology
The Medical School
Birmingham, 15

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