Studies on the Herpes Simplex Virus Alkaline Nuclease: Detection of Type-common and Type-specific Epitopes on the Enzyme

By LAWRENCE M. BANKS, IAN W. HALLIBURTON, DOROTHY J. M. PURIFOY, RICHARD A. KILLINGTON AND KENNETH L. POWELL*

MRC Herpesvirus Research Group, Department of Microbiology, University of Leeds, Leeds LS2 9JT, U.K.

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SUMMARY

Five monoclonal antibodies to the alkaline nuclease of herpes simplex virus (HSV) types 1 and 2 have been used in immunoperoxidase tests to demonstrate the nuclear localization of the enzyme within HSV-1- and HSV-2-infected cells and to purify the enzyme from cells infected with either virus by immunoadsorbant chromatography. Affinity chromatography with a \(^{32}\)P-labelled extract of HSV-2-infected cells has enabled us to demonstrate that the nuclease eluting from the immunoadsorbant is a phosphoprotein, hence confirming the nuclease to be identical to the phosphorylated polypeptide previously referred to as ICSP 22 (HSV-2) or ICP 19 (HSV-1). In addition, the results clearly demonstrate that monoclonal antibodies Q1, CC1 and CH2 are directed against HSV type-common epitopes while V1 and T2T1 antibodies are against HSV-2-specific epitopes on the enzyme. Using the type-specific monoclonal antibodies in an immunoperoxidase test, the enzyme specified in cells infected with intertypic recombinants has been typed; correlation of these data with restriction endonuclease maps of the recombinants has enabled us to map the position of the active site of the nuclease gene to map units 0.168 to 0.184 on the genomes of both HSV-1 and HSV-2. Taken with the data mapping the mRNA encoding this enzyme, the nuclease active site can be mapped to 0.168 to 0.175 on the genome. Finally, the use of monoclonal antibodies in immunofluorescence tests on infected cells has demonstrated that the nuclease is synthesized within 2 h post-infection.

INTRODUCTION

There have been a number of reports detailing the induction during lytic infection by herpes simplex virus (HSV) types 1 and 2 of a nuclease with a high pH optimum (Keir & Gold, 1963; Hoffman & Cheng, 1978; Strobel-Fidler & Francke, 1980). This enzyme is a major viral product visible as a stained band on polyacrylamide gels after electrophoresis of whole cell extracts from HSV-infected cells (Banks et al., 1983). The alkaline nuclease has both endo- and exonucleolytic activity associated with it (Hoffman & Cheng, 1979) but due to the high pH required for optimal activity in vitro, there is still uncertainty as to its role in vivo.

Francke & Garrett (1982) have described a virus with a mutation in the nuclease gene which exhibits reduced DNA synthesis at the non-permissive temperature, pointing to a possible role for the nuclease in DNA replication. To characterize its activity, we recently reported the production of monoclonal antibodies to this enzyme (Banks et al., 1983). Three monoclonal antibodies were initially described, Q1, V1 and T2T1. Here we detail the further characterization of these monoclonal antibodies together with an additional two, CH2 and CC1, also directed against the nuclease. These monoclonal antibodies have enabled us to study the polypeptide components of the enzyme using immunoadsorbant chromatography and Western blotting. In addition, the monoclonal antibodies Q1, V1 and T2T1 have demonstrated the presence of HSV type-common and HSV type-specific epitopes on the nuclease, properties which enabled us to map the position of the gene coding for the enzyme and its active site.
**METHODS**

**Cells, medium and viruses.** Human epidermal carcinoma no. 2 (HEp-2) and African green monkey kidney (Vero) cells were grown in Eagle's medium containing 10% (v/v) calf serum and 0.025% (w/v) sodium bicarbonate. The viruses used in this study were the 186 (Rawls et al., 1968) and 3345 (Sim & Watson, 1973) strains of HSV-2 and the HFEM (Watson et al., 1966), KOS (Smith, 1964) and MP (Hoggan & Roizman, 1959) strains of HSV-1. Recent clinical isolates were S23 and S28 of HSV-2 and S19 and 0-10-2 of HSV-1. Recombinant viruses used were isolated by Halliburton et al. (1977), Halliburton (1980) and Morse et al. (1978). Cells and virus were grown as described previously (Purifoy & Benyesh-Melnick, 1975).

**Infection and radiolabelling.** Infection and radiolabelling were done as reported by Powell & Courtney (1975). Briefly, batches of 1·2 × 10⁹ HEp-2 cells growing in 80 oz glass roller bottles were inoculated with virus at a multiplicity of infection of 20 p.f.u./cell. Virus was allowed to adsorb to the cells for 1 h at 37°C. At the end of the adsorption period, the remaining inoculum was decanted and the cell monolayers rinsed twice with fresh medium. Medium containing 1 μCi [³²S]methionine or 25 μCi ³²P per ml (Amersham) as indicated in the text, was then added to the monolayers. Cells were harvested at 18 h post-infection by scraping them into the medium and were subsequently collected by centrifugation at 800 g for 5 min. The cells were washed twice with phosphate-buffered saline (PBS) before being frozen at −70°C as dry pellets.

**Protein extraction.** All procedures were done at 0 to 4°C. The frozen infected cell pellets were thawed and suspended in extraction buffer [20 mM-Tris–HCl pH 7.5, 0.5 mM-dithiothreitol (DTT)] at a cell concentration of 1 × 10⁷ to 3 × 10⁷ per ml. The cells were then subjected to ultrasonic disruption and extracted with high salt as described previously (Powell & Purifoy, 1977). The extract was dialysed overnight against several changes of PBS after which it was clarified by sedimentation at 150000 g for 1 h. The supernatant fluid was then used for immunoadsorbant chromatography.

**Preparation of monoclonal antibodies.** Cells producing monoclonal antibodies to the nuclease were isolated as described previously (Banks et al., 1983). Antibody was purified from ascites fluid using DEAE–cellulose chromatography (Fahey & Horbett, 1959).

**Immunoadsorbant chromatography.** Five to 10 mg of purified monoclonal antibody was coupled to 1 g cyanogen bromide-activated Sepharose 4B (Pharmacia) according to the manufacturer’s instructions. The infected cell extract was applied to the column (0.7 x 8 cm) and allowed to recycle overnight. The column was then washed extensively with PBS and bound material eluted sequentially with 0.3 M-NaC1, 3 M-NaC1 and 3 M-potassium saline (PBS) before being frozen at −70°C as dry pellets.

**Polyacrylamide gel electrophoresis.** SDS–PAGE was done as described by Powell & Courtney (1975). The results were analysed either by autoradiography or by fluorography (Bonner & Laskey, 1974).

**ELISA.** Enzyme-linked immunosorbent assays were done as described previously (Banks et al., 1983).

**Assay for enzyme activity.** The alkaline nuclease activity was measured as reported by Purifoy & Powell (1976).

**Immunoperoxidase tests.** Nuclease-related antigens within infected cells were detected using the test described by Banks et al. (1983). Briefly, coverslips seeded with Vero cells were infected at a multiplicity of infection of 10 p.f.u./cell and 6 h after infection the cells were fixed in methanol at −20°C. Following rehydration in PBS, the cells were reacted with the monoclonal antibody for 30 min. After a series of PBS washes, rabbit anti-mouse IgG (peroxidase conjugate, Dako, Copenhagen, Denmark) was added and allowed to react for 30 min. Following further PBS washes, the peroxidase-conjugated antibody that remained was detected by addition of substrate [75 mg Hanker–Yates reagent (Polysciences, Northampton, U.K.) in 50 ml 0·1 M-Tris–HCl pH 7·5, 0·01% H₂O₂]. After 15 min of reaction the cells were washed and mounted for microscopy.

**Immunofluorescence tests.** These were performed as described for immunoperoxidase tests as far as addition of primary antibody. After a series of PBS washes, fluorescein-labelled rabbit anti-mouse IgG (Dako) was added. After 30 min the cells were washed in PBS and water, and mounted for microscopy.

**Western blotting.** The technique used was a modification of that of Towbin et al. (1979). Virus-infected cell extracts were run on a polyacrylamide gel and then electrophoretically transferred to nitrocellulose paper (0·45 μm; Schleicher & Schüll) using the Bio-Rad Trans-Blot Cell system. Transfer took place in a buffer of 40% methanol, 20% Tris–glucose at 30V overnight. The nitrocellulose was then soaked in a solution of 3% glycine, 0·9% NaCl, 10 mM-Tris–HCl pH 7·5 and 10% calf serum for 1 h at 39°C. Following extensive washing in PBS, monoclonal antibody diluted in PBS with 10% calf serum was added and allowed to react for 2 h at 20°C. Following further washes in PBS, rabbit anti-mouse IgG (peroxidase conjugate; Dako) diluted in PBS with 10% calf serum was added for 2 h. At the end of this period the paper was washed in PBS and the proteins visualized by adding substrate (as described for immunoperoxidase). The paper was then washed in PBS, water, 50% ethanol and 75% ethanol.

**Enzyme neutralization.** Neutralization of the alkaline nuclease was performed using two different antisera. Antiserum against HSV-2 nuclease was raised using purified enzyme as immunogen, whereas antisera against type 1 was raised using an extract of HSV-1-infected cells. Virus-infected cells for these tests were extracted in 20 mM-Tris–HCl pH 7·5, 5 mM-DTT. Neutralization experiments were done by incubating 20 μl of antiserum with 20 μl of infected cell extract overnight on ice. The remaining alkaline nuclease activity was then assayed.
Table 1. Isolation of CH2 and CCI monoclonal antibodies

<table>
<thead>
<tr>
<th>Polyclonal cell lines tested</th>
<th>Number of monoclones tested</th>
<th>Reaction in ELISA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyvalent</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>2 (22)</td>
<td>+</td>
</tr>
<tr>
<td>CH1, CH2, CH3</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>CCI to CC10</td>
<td>10</td>
<td>+</td>
</tr>
</tbody>
</table>

* Subtype determined by ELISA using rabbit anti-mouse IgG subtype sera (Miles Laboratories).
† Reciprocal of endpoint dilution by ELISA of selected monoclonal antibodies (as ascitic fluids).
‡ Reaction of 1/100 dilution of monoclonal antibody on purified enzyme from HSV-1 and HSV-2, and an extract of mock-infected cells.

RESULTS

Characterization of monoclonal antibodies

Characterization of the monoclonal antibodies Q1, V1 and T2T1 has already been described by Banks et al. (1983). However, two more monoclonal antibodies to the nuclease have now been obtained using the methods described previously. The properties of these additional antibodies, CH2 and CCI, are summarized in Table 1.

Immunoadsorbant chromatography

The monoclonal antibodies were coupled to CNBr-activated Sepharose for use in immunoadsorbant chromatography. Fig. 1 shows the polypeptides retained from the extracts of HSV-2-infected cells by immunoaffinity columns prepared using each of four monoclonal antibodies to the nuclease. A typical elution profile from such a column is shown in Fig. 2. This is shown to demonstrate that background level of elution was reached after each peak was detected and before elution of the next peak began. We shall only show this one profile as each immunoadsorbant profile is similar. In the subsequent discussion we have taken the conditions required for elution of DNase as an indicator of the affinity of each antibody for this protein. Immunoaffinity columns prepared using Q1 and T2T1 antibodies showed moderate affinity for the 85 000 mol. wt. (85K) polypeptide previously shown to have nuclease activity (Fig. 1 a, b). The polypeptide eluted from the columns with 3 mM-NaCl. Columns prepared using V1 and CH2 (Fig. 1 c, d) showed higher and lower affinities for the polypeptide respectively. The polypeptide required 3 M-KSCN for elution from the V1 column and only 0.3 M-NaCl for elution from the CH2 column (Fig. 1 c, d). In each case the major DNA-binding protein also elutes from each column, a phenomenon we have examined elsewhere (Vaughan et al., 1984). Enzyme activity was destroyed by our normal immunoaffinity chromatographic procedure; however, on applying the infected cell extract in DE buffer [50 mM-Tris–HCl pH 7.5, 0.5 mM-DTT, 0.2% NP40, 20% glycerol; Powell & Purifoy (1977)] rather than PBS, there is also retention of enzyme activity (data not shown). In contrast to the above results with HSV-2, when an extract from HSV-1 (strain HFEM)-infected cells was applied to the immunoadsorbants, two of the monoclonal antibodies (V1 and T2T1) failed to react with any antigen. Clearly, the Q1 affinity column (Fig. 3 a) results in purification of an 85K polypeptide which was bound with the same affinity as that observed with an HSV-2 extract. However, V1 immunoadsorbant (Fig. 3 b) and T2T1 immunoadsorbant both failed to retain antigen in a number of experiments, a result which was confirmed by other methods demonstrating the type-specificity of these monoclonal antibodies (see below). When the same experiment was performed with CCI monoclonal antibody (Fig. 3 c) an 85K mol. wt. polypeptide was observed eluting with 3 M-NaCl and 3 M-KSCN.

These results indicate that Q1 monoclonal antibody is directed against a type-common site, and V1 and T2T1 monoclonal antibodies are directed against HSV-2-specific sites on the nuclease. We have been unable to demonstrate CCI antibody reacting with HSV-2 antigens or
Fig. 1. Immunoabsorbant chromatography of an extract of HSV-2-infected cells was done as described in Methods using (a) Q1, (b) T2T1, (c) V1 and (d) CH2 monoclonal antibody. The polypeptides eluted from the adsorbant by the following salts were analysed by PAGE and autoradiography. (a) Lane 1, material applied to column; lane 2, material not bound to column; lane 3, 0.3 M-NaCl eluate; lane 4, 3 M-NaCl eluate. (b) Lane 1, material applied to column; lane 2, 0.3 M-NaCl eluate; lane 3, 3 M-NaCl eluate. (c) Lanes 1 and 2 as in (a); lane 3, 3 M-NaCl eluate; lane 4, 3 M-KSCN eluate. (d) Lane 1, material applied to column; lane 2, 0.3 M-NaCl eluate. The upper arrow in each case indicates the major DNA-binding protein, the lower the alkaline nuclease. Each protein could be unequivocally identified by Western blotting.
HSV alkaline nuclease

Fig. 2. Immunoadsorbant chromatography using monoclonal antibody CC1. A sample of $^{35}$S-methionine-labelled infected cell extract was applied to the column as described in Methods followed by washing with loading buffer, 0.3 M-NaCl, 3 M-NaCl and 3 M-KSCN. In each case washes were continued until background levels of radioactivity were obtained.

CH2 antibody reacting with HSV-1 antigens by immunoadsorbant chromatography; however, these antibodies have been shown to be type-common by ELISA (Table 1), immunoperoxidase and Western blotting (see below).

HSV phosphoproteins

Several HSV-induced polypeptides are phosphorylated (Pereira et al., 1977; Marsden et al., 1978; Wilcox et al., 1980). One such polypeptide corresponds to ICP 19, which is believed to be the functional polypeptide of the alkaline nuclease (Banks et al., 1983). To confirm whether the enzyme was phosphorylated, immunoadsorbant chromatography was performed with Q1 monoclonal antibody using a $^{32}$P-labelled extract of HSV-2-infected cells. The results of this experiment are shown in Fig. 4. The 85K polypeptide eluting with 3 M-NaCl was highly phosphorylated and when this material was compared to the extract applied to the column, it is apparent that the antigen represents a major phosphoprotein in HSV-2-infected cells.

Determination of time of synthesis of the alkaline nuclease

The procedure for detection of HSV-induced enzymes has generally been based on assays of enzyme activity. Here, immunofluorescence using the monoclonal antibodies reacted with cells at various times after infection with HSV-1 and HSV-2 has been used in attempts to define more accurately the time at which synthesis of the alkaline nuclease begins. Fig. 5 shows the results of immunofluorescence with Q1 monoclonal antibody on cells infected for 0 to 6 h with HSV-2. Clearly, the presence of antigen can be observed within the nuclei of cells by 2 h post-infection. Changes in the pattern of fluorescence within the nuclei were observed throughout this time period, appearing to indicate specific localization of the enzyme within certain areas of the nucleus. When the experiment was performed on HSV-1-infected cells there was a noticeable delay of approximately 1 h in appearance of the enzyme within the nuclei, confirming previous data demonstrating the slower induction of protein synthesis as well as host shut-off, in HSV-1-infected cells (Powell et al., 1977). Fig. 6 shows the results observed when CH2 monoclonal antibody is used on cells 4 to 6 h post-infection. This demonstrated that CH2 monoclonal antibody fails to react with the cells until 6 h post-infection, indicating that the epitope to which this monoclonal antibody reacts was not available on the nuclease until this time. This result was not due to simple lack of potency of CH2 antibody since diluting Q1 antibody and reacting it with the same cells did not cause the same result.
Fig. 3. Immunoabsorbant chromatography of an extract of HSV-1-infected cells was done as described in Methods using (a) Q1, (b) V1 and (c) C3 monoclonal antibodies. The polypeptides eluted from the adsorbant by the following salts were analysed by PAGE and autoradiography. Lanes 1 to 4 in each of (a), (b) and (c) represent: 1, material applied to column; 2, material not bound by column; 3, material eluted with 0·3 M-NaCl; 4, material eluted with 3 M-NaCl; lanes 3 and 4 in (c) represent 3 M-NaCl and 3 M-KSCN eluates respectively.
Fig. 4. Immunoadsorbant chromatography of an extract of $^{32}$P-labelled, HSV-2-infected cells was done as described in Methods. Polypeptides present in each fraction were analysed by PAGE and autoradiography. Lane 1 represents material applied to the column and lane 2 material not bound by the column; lanes 3 and 4 and 5 and 6 represent the 0.3 and 3 M-NaCl eluates respectively; lane 7 is a stained gel showing the polypeptides present in lane 5.

Polypeptide constituents and strain variation in the alkaline nuclease

Purified preparations of the nuclease from HSV-infected cells contain several polypeptides (Strobel-Fidler & Franke, 1980; Banks et al., 1983). Alkaline nuclease purified from HSV-2-infected cells consists of one major polypeptide species of 85K; however, when the enzyme is purified from HSV-1 infected cells, a major polypeptide of 85K and a minor polypeptide of 80K are presented in these preparations (Banks et al., 1983).

To investigate further the polypeptide constituents of the enzyme and strain variation in the alkaline nuclease, we have used Western blotting with the monoclonal antibodies on a number of HSV strains. Extracts from cells infected with several laboratory-established HSV strains or recent clinical isolates were run on polyacrylamide gels. Following blotting onto nitrocellulose paper, these were reacted with the monoclonal antibodies as described in Methods and the results obtained are shown in Fig. 7. Monoclonal antibodies Q1, CH2 and CC1 (Fig. 7a, b, c) clearly reacted with all six HSV strains used, although CC1 appeared to react better with the enzyme from HSV-1 strains whereas CH2 had a greater reaction with the HSV-2 strain 186 enzyme. These results, however, also demonstrated considerable molecular weight variation in the polypeptide between different HSV strains. The difference in molecular weight of the alkaline nuclease between the virus strains is as much as 2K to 3K as indicated by the results with the 186 and HFEM strains. In addition, the Q1 monoclonal antibody reacted with two minor polypeptide species of approximately 90K and 80K from HSV-1 strains. Q1 occasionally detected small molecular weight polypeptides (Fig. 7, lanes 3 and 6); however, these were not reproducible and probably represent degradation products. CC1 also appeared to react with the
Fig. 5. Alkaline nuclease detected in virus-infected cells using a monoclonal antibody (Q1) and fluorescein-labelled rabbit anti-mouse immunoglobulin serum. Cells were infected with HSV-2 for 0, 1, 2, 3, 4, 5 or 6 h as indicated and were then fixed and stained as described in Methods. Staining was observed using a Vickers microscope fitted with a suitable light source. Nuclease was first detected from 1 to 2 h post-infection.

minor polypeptide of 90K. Clearly, there were at least three high molecular weight polypeptides of about 80K, 85K and 90K which shared antigenic sites. From Fig. 7(d) it can be seen that V1 monoclonal antibody only reacted with HSV-2 extracts, confirming that this monoclonal antibody is directed against an HSV-2 specific epitope on the alkaline nuclease.
monoclonal antibody (Fig. 7e) only reacted with HSV-2 strain 186, demonstrating in this experiment that the antibody recognized a strain-specific site. However, when 50 additional strains were used, T2T1 antibody was shown to react with approximately 80% of HSV-2 strains (S. Riddington, personal communication). None of the antibodies reacted with mock-infected cells.

Mapping of the alkaline nuclease using intertypic recombinants

Based on the type-specificity of the monoclonal antibodies, immunoperoxidase tests on a series of intertypic recombinants have enabled us to map the position of the alkaline nuclease on the HSV genome. Vero cells were infected with HSV recombinant viruses and their parental strains. Fig. 8 shows the results of immunoperoxidase tests on cells infected with HSV strains HFEM (type 1), and 186 (type 2). Clearly monoclonal antibodies T2T1 and V1 only reacted with HSV-2-infected cells, whereas Q1 monoclonal antibody reacted with both HSV-1- and HSV-2-infected cells. Table 2 shows the results obtained for the exonuclease serotype of 17 intertypic recombinants as determined by immunoperoxidase tests and neutralization of the enzyme activity using type-specific sera. It is apparent from these results that the two sets of data are in complete agreement. Utilizing the map positions of the recombinant viruses (Fig. 9) it is clear that RB29 and RB210 are the crucial recombinants. RB210 produces a type 1 nuclease, defining the lower limit as 0.168, whereas RB29 produces type 2 nuclease, hence defining the upper limit as 0.184 and this therefore maps the position of the alkaline nuclease active site on the genomes of both HSV-1 and HSV-2. The results with all other recombinants are compatible with this conclusion.
Fig. 7. Detection of alkaline nuclease within infected cells by Western blotting. Monoclonal antibodies used were (a) Q1, (b) CH2, (c) CC1, (d) V1 and (e) T2T1. Strains of virus used to infect cells were: lanes 1, S23; lanes 2, S28; lanes 3, 186 (all HSV-2 strains); lanes 4, 0-10-2; lanes 5, S19; lanes 6, HFEM (all HSV-1 strains). Western blotting was done as described in Methods.
Fig. 8. Detection of alkaline nuclease within infected cells by the indirect immunoperoxidase test. Cells were infected for 6 h with HSV-1 (a) or HSV-2 (b). Nuclease within infected cells was then detected by antibodies Q1, V1, T2T1 or preimmune serum (PI) as described in Methods.

**DISCUSSION**

Five monoclonal antibodies to the HSV alkaline nuclease have been isolated and used in immunoabsorbant chromatography to purify an 85K polypeptide. This polypeptide eluting
Fig. 9. Maps of DNA contained in the genomes of HSV-1 × HSV-2 recombinants important in the mapping of the nuclease gene. The thick upper line represents the position on the conventional map of the HSV genome (from 0 to 0-3 units). The upper (dotted) line represents HSV-1 DNA, the lower solid line HSV-2 DNA. Regions of uncertainty are indicated by sloping dotted lines. RB<sub>29</sub> and RB<sub>210</sub> are the critical recombinants specifying nuclease of alternate types and differing only in the region 0-168 to 0-184 in DNA content.

Table 2. Reaction of monoclonal antibodies with intertypic recombinants

<table>
<thead>
<tr>
<th>Recombinant</th>
<th>Nuclease type (neutralization)</th>
<th>Reaction of monoclones*</th>
<th>Nuclease type (monoclones)</th>
</tr>
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<tbody>
<tr>
<td>D3E1</td>
<td>2</td>
<td>++ ++ + + + + + + + + +</td>
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</tr>
<tr>
<td>BIE</td>
<td>2</td>
<td>++ ++ + + + + + + + + +</td>
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</tr>
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<td>A6D</td>
<td>2</td>
<td>++ ++ + + + + + + + + +</td>
<td>2</td>
</tr>
<tr>
<td>D5E1</td>
<td>2</td>
<td>++ ++ + + + + + + + + +</td>
<td>2</td>
</tr>
<tr>
<td>D4E3</td>
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<td>++ ++ + + + + + + + + +</td>
<td>2</td>
</tr>
<tr>
<td>D1E5</td>
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<td>D4E1</td>
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<td>+ + + ++ + + + + + + + +</td>
<td>2</td>
</tr>
<tr>
<td>C5D</td>
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<td>+ + + + + + + + + + + +</td>
<td>2</td>
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<td>D1E4</td>
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<td>++ ++ + + + + + + + + +</td>
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<td>KOS</td>
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<td>186</td>
<td>2</td>
<td>+ + + + + + + + + + + +</td>
<td>2</td>
</tr>
</tbody>
</table>

* + indicates the intensity of nuclear staining observed.

from the columns retains good antigenic and some enzymic activity, indicating that purification of enzyme via immunoabsorbant chromatography has no overt deleterious effect on the polypeptide. The purification of the enzyme by this procedure represents a significant improvement on other published methods in the speed of purification (Strobel-Fidler & Francke, 1980; Banks et al., 1983).
The data presented demonstrate a range of antigenic determinants present on the alkaline nuclease. Q1 monoclonal antibody clearly reacts with a highly conserved site on the enzyme, a fact which is probably not too surprising since this antibody is capable of neutralizing the enzyme activity (Banks et al., 1983). CC1 monoclonal antibody appears also to react with a type-common site on the alkaline nuclease, but it clearly reacts much better with HSV-1 than HSV-2 strains. The results obtained with the CH2 monoclonal antibody appear at first sight to be somewhat strange since although the antibody was obtained using spleen cells from mice immunized with HSV-1 strain HFEM alkaline nuclease, it reacts best with the 186 strain enzyme. We do not have an explanation for this result but one explanation might be an epitope masked in most strains but more available in the 186 enzyme. Such an epitope may be exposed by whatever mechanism causes the change in size of the polypeptide.

V1 monoclonal antibody is clearly directed against an epitope on the nuclease which is HSV-2-specific, whereas T2T1 monoclonal antibody which is type-specific also appears to exhibit some strain specificity. This demonstrates that the epitope defined by T2T1 is absent in some HSV-2 strains and that Q1, V1 and T2T1 all react with different epitopes.

The use of Q1 monoclonal antibody to detect the alkaline nuclease in HSV-infected cells clearly demonstrates that the enzyme is present within the nucleus by 2 h post-infection. At this time, the enzyme appears to be rather diffuse within the nucleus, but it is noticeable that as the infection progresses, the nuclease becomes localized in 'globular' structures. The results with CH2 monoclonal antibody are interesting in that this seems to indicate a change in the antigenic structure of the nuclease at around 5 to 6 h post-infection, since CH2 fails to react with infected cells prior to this time. This raises the possibility that the nuclease may undergo some form of previously undetected post-translational modification and this is now under further investigation.

Western blot analysis of HSV-infected cell extracts with the monoclonal antibodies raises a number of interesting points. Firstly, the results with Q1 and CC1 antibodies appear to indicate the presence of polypeptides antigenically related to the alkaline nuclease of about 80K and 90K. The lower molecular weight polypeptide becomes apparent during purification of HFEM nuclease (Banks et al., 1983; Francke & Garrett, 1982) and was thought to be a degradation product, but the 90K polypeptide detected by the antibodies was unexpected and at present its significance is unknown. However, it has been demonstrated that a 1.9 kb mRNA, whose translation product has not been identified, overlaps with the mRNA encoding the alkaline nuclease (Costa et al., 1983), hence raising the possibility that this mRNA may be responsible for one of these polypeptides. Secondly, the results clearly demonstrate considerable variation in the molecular weight of the functional polypeptide of the nuclease between HSV strains. The highly variable nature of the polypeptide now known to have the nuclease activity was first noted by Powell et al. (1977). The mechanism causing this variation is of some interest and we will investigate this further. We have noticed only much more minor variation in the size of other HSV enzymes (Banks et al., 1984). Using the type-specificity of the monoclonal antibodies in the immunoperoxidase test has enabled us to map the position of the nuclease on the genomes of both HSV-1 and HSV-2 to 0.168 to 0.184. This corresponds closely to the values obtained by Preston & Cordingley (1982) and Costa et al. (1983) as determined by translation in vitro. The map position determined by Costa et al. (1983) for the mRNA coding for the HSV-1 alkaline nuclease was 0.164 to 0.175. Because V1 monoclonal antibody is HSV-2-specific, this maps the V1 epitope to 0.168 to 0.175, which also possibly represents the map position of the active site of the enzyme, since the polyclonal antibodies used neutralized enzyme activity.

Further work now involves isolation of mutants with defects in the enzyme in an attempt to obtain more data concerning the actual role of the nuclease in infection. In addition, the monoclonal antibodies are being used to study possible protein complexes involving the nuclease within infected cells.

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