Comparative Studies on LS Antigens Induced by Vaccinia and Cowpox Viruses

(Accepted 26 July 1979)

SUMMARY

The constituents of LS antigen from cells infected with vaccinia virus and with cowpox virus were compared by immunoprecipitation and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Antiserum to the LS antigen from cells infected with vaccinia virus reacted with at least five polypeptides in cells infected with either virus. Four of these polypeptides were similar sizes in cells infected with the two viruses. However, one major polypeptide with a mol. wt. of about 100,000 (100 K) detected in cells infected with vaccinia virus was not found in cells infected with cowpox virus. Conversely, a polypeptide with a mol. wt. of about 60,000 (60K) was detected only in cells infected with cowpox virus.

Vaccinia and cowpox viruses belong to the same genus, Orthopoxvirus, of the Poxviridae. These viruses cannot be distinguished from each other by the complement fixation test (Macdonald & Downie, 1950; Downie & Dumbell, 1956) but on immunodiffusion, one major precipitable component of vaccinia-soluble antigen was scarcely detectable in cowpox-soluble antigen (Gispen, 1955; Rondle & Dumbell, 1962). However, Rondle & Dumbell (1962) showed that antiserum to cowpox precipitates this major component from vaccinia soluble antigen. During attempts to identify the LS antigen in cells infected with vaccinia virus and in those infected with cowpox virus, we found that the precipitable antigen reported by Gispen (1955) and Rondle & Dumbell (1962) was a major component of the LS antigen fraction of vaccinia virus-infected cells, but that it was not produced in cells by cowpox virus infection.

RK-13 cells derived from rabbit kidney cells, purchased from Flow Laboratories, were grown in minimum essential medium (MEM; Eagle, 1959) supplemented with 10% calf serum. The LS antigen fraction was prepared from RK-13 cells infected with the Lister strain of vaccinia virus by the method of Craigie & Wishart (1936) with slight modifications (Cohen & Wilcox, 1966). Antiserum to this LS antigen fraction, named anti-LS(W), was prepared by immunizing rabbits with three intramuscular injections of this antigen suspended in Freund's adjuvant. On immunodiffusion of the extracts of vaccinia virus-infected RK-13 cells with this anti-LS(W) serum, several precipitin lines with one major line were obtained (data not shown). The region of the gel containing the major component, apparently free from contamination with other components, was then carefully excised and thoroughly washed with phosphate-buffered saline, pH 7.4. Rabbits were immunized as described above with the gel containing this major component and the antiserum obtained was named anti-LS(M). This anti-LS(M) serum recognized only the major component with anti-LS(W) serum. These antisera were then used to identify the antigenic polypeptide in the LS antigen fraction by immunoprecipitation and SDS-PAGE.

The Lister strain of vaccinia virus and the LB red strain of cowpox virus, purified from infected RK-13 cells, were used as stock viruses for inoculation after titration of infectivity by the plaque test. The methods for virus purification and infectivity titration were described previously (Ikuta et al. 1978a). Monolayer cells of RK-13 were infected at a m.o.i. of 10...
Fig. 1. Electropherograms of immunoprecipitates with anti-LS serum. RK cells were infected at 10 p.f.u./cell with vaccinia virus (VV) or cowpox virus (CV), or were mock-infected (M). They were then labelled for 24 h with $^{14}$C-leucine in the presence (+) or absence (−) of Ara C. The labelled cells were solubilized in TD buffer, and then centrifuged at 100,000 g for 2 h. The resulting supernatants were treated with (a) anti-LS(W) serum or (b) anti-LS(M) serum and the immunoprecipitates were analysed by SDS-PAGE and fluorography. The following standard proteins were also subjected to SDS-PAGE in the same gel to estimate the mol.wt. of antigenic polypeptides: BDH mol.wt. marker mixtures (product No. 44223 2U, mol.wt. range 14,300 to 71,500, and 44230 2R, mol.wt. range 53,000 to 265,000): myosin, mol.wt. 215,000; bovine serum albumin, mol.wt. 67,000; ovalbumin, mol.wt. 43,500 and cytochrome c, mol.wt. 12,400.

or were mock-infected, in the presence or absence of cytosine-1-β-D-arabinofuranosyl-HCl (Ara C; 50 μg/ml), which is an inhibitor of DNA synthesis. After adsorption for 1 h at 37 °C, the infected cells were labelled with 2 μCi/ml of $^{14}$C-leucine (297 mCi/mmol; New England Nuclear) in MEM containing one-tenth the normal concentration of leucine and 2 % dialysed calf serum for 24 h in the presence or absence of Ara C. A cell extract was prepared from these labelled cells, as reported previously (Ikuta et al. 1978b). Briefly, the labelled cells were solubilized with TD buffer (1 % Triton X-100, 0.5 % sodium deoxycholate, 0.15 M-NaCl, 1 mM-phenylmethylsulphonyl fluoride, 10 mM-sodium phosphate buffer, pH 7.2, by sonication for 3 min and the mixture was centrifuged at 100,000 g for 2 h at 4 °C. The cell extract was incubated with anti-LS(W) or anti-LS(M) serum and then the immune complexes were precipitated with staphylococcal (Cowan I strain) protein A-adsorbent by the method of Kessler (1975) with slight modifications (Ikuta et al. 1979). The immune complexes were washed three times with TD buffer and once with 10 mM-sodium phosphate buffer, pH 7.2. The immune complexes were eluted from the bacterial adsorbent by heating
for 3 min in ‘sample buffer’ and then subjected to SDS-PAGE. The composition of the ‘sample buffer’ and the conditions for SDS-PAGE were as described previously (Ikuta et al. 1978a). After electrophoresis, fluorography was carried out by the method of Bonner & Laskey (1974).

Fig. 1 shows the fluorograms of SDS-PAGE of immunoprecipitates with anti-LS(W) serum and anti-LS(M) serum. As shown in Fig. 1(a), anti-LS(W) serum reacted with at least five components in cells infected with vaccinia virus. Of these components, the three with low mol. wt. were virus-induced early polypeptides synthesized even in the presence of Ara C and the other two components with high mol. wt. were virus-induced late polypeptides synthesized only in the absence of Ara C. These observations were consistent with the finding that the LS antigen fraction contains two distinct classes of polypeptides: low mol. wt. components synthesized early in the course of infection and high mol. wt. components synthesized later in the course of infection (Cohen & Wilcox, 1966; Wilcox & Cohen, 1967). In cells infected with cowpox virus also, at least five components reacted with the anti-LS(W) serum. However, one major late polypeptide with a mol. wt. of about 100,000 (100K) synthesized in vaccinia virus infected cells was not detectable in cowpox virus-infected cells and a polypeptide with a mol. wt. of about 60,000 (60K) was detected as an early polypeptide in cowpox virus-infected cells, but was not detected in vaccinia virus-infected cells. The other three early components and one late component were detected as polypeptides comparable in size to those in cells infected with vaccinia virus.

On immunoprecipitation, anti-LS(M) serum recognized only the 100M polypeptide in vaccinia virus-infected cells and only the 60K polypeptide in cowpox virus-infected cells (Fig. 1b). Marquardt et al. (1969) showed that the e-f precipitinogen with a mol. wt. of 96,600 has some immunological and chemical properties attributed to the LS antigen of vaccinia virus. This result suggests that the 100K polypeptide is the e-f antigen and therefore the LS antigen.

Fig. 2 shows the fluorograms of immunoprecipitates with rabbit antiserum against vaccinia virus (anti-vaccinia) and against cowpox virus (anti-cowpox). The antisera were obtained as described previously (Miyamoto & Kato, 1971). Briefly, each virus was grown in RK-13 cells and then injected into the skin of the back of rabbits; immune sera were obtained 2 months after virus inoculation. The soluble antigens for immunoprecipitation were the same as those used in the experiments for Fig. 1. Both cells infected with vaccinia virus and those infected with cowpox virus contained about 30 polypeptides that reacted with anti-vaccinia or anti-cowpox serum. Most of the antigenic polypeptides in vaccinia virus-infected cells were similar to those in cowpox virus-infected cells. However, the 100K polypeptide was detected only in vaccinia virus-infected cells as one of the major components reacting with anti-vaccinia and also with anti-cowpox serum. On the other hand, the 100K polypeptide was not detected with anti-vaccinia or anti-cowpox serum in cowpox virus-infected cells, while the 60K polypeptide was detected in cowpox virus-infected cells with both antisera. Rondle & Dumbell (1962) also found by gel-diffusion studies that component f was readily demonstrable as a soluble precipitable substance in cells infected with vaccinia virus, but was scarcely detectable in cells infected with cowpox virus and that anti-f antibody was readily detectable in antisera prepared against cowpox and vaccinia. Further work showed that the component f was LS (Rondle & Williamson, 1968). Thus, the results shown in Fig. 1(b) and Fig. 2 suggest that the antigenic determinant of the 100K polypeptide in vaccinia virus-infected cells is the same as that of the 60K polypeptide in cowpox virus-infected cells, although the sizes of the polypeptides and the effect of Ara C on their synthesis are apparently different.

A late polypeptide at the top of the gel which reacted with anti-vaccinia and anti-cowpox
sera was demonstrated in cells infected with vaccinia virus, but not in those infected with cowpox virus. However, this polypeptide did not react with either anti-LS(W) or anti-LS(M), indicating that it was not related to the LS antigen.

The 100K polypeptide, which is a major antigenic component in vaccinia virus-infected cells, was not found in cowpox virus-infected cells. These results indicate that cowpox virus and vaccinia virus are distinguishable by the size of a major component of LS antigen, which is a typical major group of antigens induced by poxvirus infection, although both viruses belong to the same genus, Orthopoxvirus, of the Poxviridae.

This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

Department of Pathology  
Research Institute for Microbial Diseases  
Osaka University, Suita  
Osaka 565, Japan

K. Ikuta  
H. Miyamoto*  
S. Kato

* Present address: Department of Microbiology, Wakayama Medical College, Wakayama 640, Japan.
REFERENCES


(Received 10 April 1979)