Biochemical Studies on Early Cell Surface Antigen Induced by Vaccinia and Cowpox viruses

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SUMMARY

The early cell surface antigen (ECSA) induced by vaccinia or cowpox virus infection was analysed by immunoprecipitation and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The components with a mol. wt. of about 40000 (40 K) in vaccinia virus-infected cells and with a mol. wt. of about 43000 (43 K) in cowpox virus-infected cells were specifically immunoprecipitated with anti-ECSA serum prepared by immunizing rabbits with rabbit kidney cells infected with a conditional lethal mutant. The 40 K and 43 K polypeptides were synthesized even in the presence of cytosine-1-β-D-arabinofuranosyl-HCl (Ara C) and were detectable even in the cells pulse-labelled between 1 and 2 h p.i.

The appearance of cell surface antigen (CSA) in poxvirus-infected cells was demonstrated by immunofluorescence and immune haemadsorption (Miyamoto & Kato, 1968, 1971). Recently, we demonstrated by immunoprecipitation that the 64 K and 38 K polypeptides were the CSAs induced by Shope fibroma virus infection (Ikuta et al. 1979a). In cells infected with vaccinia or cowpox virus, the CSA was also detected as ECSA induced before virus DNA synthesis (Miyamoto & Kato, 1968, 1971; Ueda et al. 1969, 1972). In this work, we have characterized this ECSA by immunoprecipitation and SDS-PAGE, using a conditional lethal mutant, the DI3 strain of vaccinia virus, isolated by Tagaya et al. (1961).

The DI3 strain can grow and produce plaques in chick embryo fibroblasts (CEF), but it cannot multiply in RK13 cells derived from rabbit kidney cells, inducing only ECSA (Ueda & Tagaya, 1973). The DI3 strain, which was kindly supplied by Dr I. Tagaya, National Institute of Health, Japan, was grown on CEF. The following poxviruses were grown on RK13 cells: the IHD-W, IHD-J and Lister strains of vaccinia virus; the LB red strain of cowpox virus; and the OA strain of Shope fibroma virus. The IHD-W and IHD-J strains were kindly supplied by Dr Y. Ichihashi, Niigata University, Japan. CEF and RK13 cells were cultured as described previously (Ikuta et al. 1978a). The stock viruses used for inoculation were purified by sucrose gradient centrifugation as described by Joklik (1962) with slight modifications (Ikuta et al. 1978a). The infectivity of stock viruses was assayed by plaque- or focus-formation as described previously (Ikuta et al. 1978a).

Monolayers of RK13 cells were infected at a multiplicity of 10 with vaccinia virus, cowpox virus or Shope fibroma virus, or mock-infected, in the presence or absence of Ara C (50 μg/ml). After adsorption for 1 h at 37 °C, the cells were washed three times with phosphate-buffered saline, pH 7.4 (PBS) and then labelled with 3 μCi/ml of 35S-methionine (442.1 Ci/mmol; New England Nuclear, Boston, U.S.A.) in Eagle's minimum essential medium (MEM) containing one-tenth the normal concentration of methionine and 2% dialysed calf serum for 24 h at 37 °C in the presence or absence of Ara C. These 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) and centrifuged in a Beckman SW50.1 rotor at 100,000 g for 2 h at 4 °C, as described previously (Ikuta et al. 1978b). The resulting supernatant (termed TD-lysate), in which
about 70% of the trichloroacetic acid-insoluble radioactivity of whole cells was consistently found, was subjected to immunoprecipitation. Indirect immunoprecipitation was carried out by the method of Kessler (1975) with slight modifications (Ikuta et al. 1979b). The Cowan I strain of Staphylococcus aureus was used in place of the second antibody. The immune complexes were separated from the bacterial absorbent in ‘sample buffer’ for SDS-PAGE (1% SDS, 1% 2-mercaptoethanol, 10% glycerol, 0.001% phenol red, 50 mM-tris-HCl buffer, pH 8.2). SDS-PAGE was conducted as described previously (Ikuta et al. 1978a), except that the gel was treated with 2, 5-diphenyloxazole to obtain the fluorogram, as described by Bonner & Laskey (1974). Fig. 1(a) shows the SDS–PAGE patterns of immunoprecipitates with rabbit antiserum against cowpox virus (anti-cowpox). Anti-cowpox serum was obtained as described previously (Miyamoto & Kato, 1971). Briefly, rabbits were inoculated with cowpox virus grown on RK13 cells. Immune serum was obtained 2 months after virus inoculation. Samples from cells cultured in both the absence and presence of Ara C gave relatively similar profiles after infection with the Lister strain of vaccinia virus and cowpox virus, except for a few polypeptide bands specific to vaccinia or cowpox virus. The profiles of immunoprecipitates from cells infected with the IHD–W strain or with the IHD–J strain were similar to those with the Lister strain (data not shown). On the other hand, in TD-lysates of RK13 cells infected with the DIIs strain of vaccinia virus, only two antigenic polypeptides were detectable with anti-cowpox serum, and these polypeptides were synthesized even in the presence of Ara C. The mol. wt. of these two polypeptides, calculated as described in the legend to Fig. 1, were about 40000 and 32000. These two polypeptides were tentatively designated as 40 K and 32 K, respectively. Only one polypeptide with a mol. wt. of about 64000 (64 K) was detected with anti-cowpox serum in a TD-lysate of Shope fibroma virus-infected cells cultured in the absence of Ara C. This 64 K polypeptide seems to be ‘nucleoprotein antigen’ cross-reactive between Orthopoxvirus and Leporipoxvirus, as described previously (Ikuta et al. 1979b). None of these components was detectable in TD-lysates from mock-infected cells.

Thus the DIIs strain induces only two virus-induced antigenic polypeptides in RK13 cells and these are synthesized even in the presence of Ara C. The following experiments using antiserum against ECSA (anti-ECSA) were performed to determine which of these two polypeptides induced in RK13 cells by infection with the DIIs strain is the ECSA polypeptide. Anti-ECSA serum, obtained from rabbits by injecting partially purified ECSA from RK13 cells infected with the DIIs strain (Ueda et al. 1972), was also kindly supplied by Dr I. Tagaya. This anti-ECSA serum specifically reacted by immunofluorescence with ECSA, but not with the vaccinia-specific antigens in the cytoplasm of acetone-fixed infected cells (Ueda et al. 1972). Fig. 1(b) shows the SDS–PAGE patterns of the immunoprecipitates with anti-ECSA serum. The same TD-lysates as those for Fig. 1 were used as soluble antigen for immunoprecipitation with anti-ECSA serum. The polypeptide with a mol. wt. of about 40000 (40 K) was detected in cells infected with the IHD-W, IHD-J or DIIs strain of vaccinia virus. On the other hand, the polypeptide with a mol. wt. of about 43000 (43 K) was detected in cells infected with cowpox virus. These 40 K and 43 K polypeptides were synthesized even in the presence of Ara C. However, the 40 K polypeptide reactive with anti-ECSA serum was not detected in cells infected with the Lister strain. None of the components of TD-lysates of cells infected with Shope fibroma virus, or of mock-infected cells reacted with anti-ECSA serum.

Immunoprecipitation tests were made in the same way on CEF infected with the DIIs strain and labelled with 35S-methionine (Fig. 1c and d). SDS–PAGE analysis of the immunoprecipitates with anti-cowpox serum showed that many antigenic polypeptides were produced in CEF by DIIs strain infection (Fig. 1c). The profiles were similar to those of
Fig. 1. SDS-PAGE analysis of immunoprecipitates with anti-cowpox serum or anti-ECSA serum. RK13 cells (a and b) or CEF cells (c and d) were infected with the IHD-W (W), IHD-J (J), Lister (L) or Dis (D) strain of vaccinia virus, cowpox virus (CV), Shope fibroma virus (SV), or mock-infected (M), in the presence (+) or absence (−) of Ara C. The infected cells were labelled with 35S-methionine for 24 h. TD-lysates prepared from the labelled cells were treated with anti-cowpox serum (a and c) or anti-ECSA serum (b and d), and the immunoprecipitates were analysed by SDS-PAGE and fluorography. In this and all succeeding electropherograms, the bottom of the figure is the anodal side of the gel. 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 mixture (product no. 44223 2U, mol. wt. range 14,300 to 71,500) purchased from BDH Chemicals Ltd, Poole, Dorset; bovine serum albumin, mol. wt. 67,000; ovalbumin, mol. wt. 43,500; and cytochrome c, mol. wt. 12,400.
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Fig. 2. Time course of production of the 40 K polypeptide in RK13 cells infected with the Dis strain. RK13 cells were infected with the Dis strain (I), or mock-infected (M), in the presence (+) or absence (−) of Ara C. After adsorption for 1 h, the infected cells were cultured with normal medium in the presence or absence of Ara C. At the times indicated in the figure (p.i.), the infected cells were labelled with 35S-methionine for 1 h at 37°C, in the presence or absence of Ara C. TD-lysates were prepared from the pulse-labelled cells and subjected to immunoprecipitation with anti-ECSA serum. The immunoprecipitates were analysed by SDS–PAGE and fluorography. The mol. wt. of the polypeptide was calculated as described in the legend to Fig. 1.

immunoprecipitates of RK13 cells infected with the Lister strain of vaccinia virus, as shown in Fig. 1(a). However, anti-ECSA serum reacted only with the 40 K polypeptide in the same TD-lysate of CEF infected with the Dis strain as that used for Fig. 1(c) (Fig. 1d). These results indicate that the anti-ECSA serum used here is monospecific against virus-induced early antigenic polypeptide.

The existence of two types of ECSA (major ECSA and minor ECSA) in vaccinia virus-infected cells was demonstrated by mixed agglutination by Ito & Barron (1972). Moreover, it was shown that the Lister strain of vaccinia virus was defective in the major ECSA, but could produce the minor ECSA (Ito & Barron, 1972; Amano et al. 1979). In our immunoprecipitation experiment, we could not detect the 40 K polypeptide reactive with anti-ECSA serum in cells infected with the Lister strain, although cells infected with other strains of vaccinia virus contained the 40 K polypeptide reactive with anti-ECSA serum (Fig. 1b). Moreover, Ito & Barron (1972) showed that antiserum against ECSA, prepared in the same way from rabbit kidney cells infected with the Dis strain, reacted only with the major ECSA, and we also showed that the anti-ECSA serum used here was monospecific (Fig. 1d). The 43 K polypeptide was also detected with this anti-ECSA serum in cells infected with
cowpox virus. These observations indicate that the 40 K and 43 K antigenic polypeptides are the major ECSA in vaccinia virus-infected and cowpox virus-infected cells, respectively. Although the size of ECSA in cells infected with cowpox virus differs from that of ECSA in cells infected with vaccinia virus, the two antigenic polypeptides may carry the same antigenic determinants, since anti-ECSA serum prepared from cells infected with the DIs strain reacted with only the 40 K polypeptide in cells infected with vaccinia virus, and with only the 43 K polypeptide in cells infected with cowpox virus (Fig. 1 b). Thus, the 40 K polypeptide is cross-reactive with the 43 K polypeptide. On the other hand, Fig. 1 (a) shows that the TD-lysates of cells infected with the Lister strain and of those infected with cowpox virus, which had been cultured in the presence of Ara C, contained the 40 K and the 43 K polypeptides, respectively, reactive with anti-cowpox serum. However, none of the components in the Lister-TD-lysate reacted with anti-ECSA serum, although the 43 K polypeptide in the cowpox-TD-lysate was precipitated with anti-ECSA serum (Fig. 1 b). This may be explained by the idea that the 40 K polypeptide reactive with anti-ECSA serum is part of the 40 K-sized polypeptides reactive with anti-cowpox serum and therefore that the 40 K polypeptide reactive with anti-ECSA serum is not produced by infection with the Lister strain, although the other 40 K-sized polypeptides are induced.

The 32 K antigenic polypeptide, detected in RK13 cells infected with the DIs strain as shown in Fig. 1 (a), is distinct from the major ECSA since it did not react with the anti-ECSA serum used here (Fig. 1 b).

We also analysed the time course of production of the 40 K polypeptide using pulse-labelled cells. RK13 cells were mock-infected or infected with the DIs strain at 10 p.f.u./cell in the presence or absence of Ara C (50 μg/ml) and adsorbed for 1 h at 37 °C. Then the infected cells were cultured in MEM supplemented with 2 % dialysed calf serum in the presence or absence of Ara C. At appropriate times (see legend to Fig. 2), they were labelled with 3 μCi/ml of 35S-methionine in methionine-free MEM supplemented with 2 % dialysed calf serum for 1 h at 37 °C in the presence or absence of Ara C. TD-lysates were prepared from the pulse-labelled cells and allowed to react with anti-ECSA serum, as described above. The immunoprecipitates were analysed by SDS-PAGE and fluorography (Fig. 2). The 40 K polypeptide was detectable in cells infected with the DIs strain and pulse-labelled between 1 and 2 h p.i. This 40 K polypeptide was detected even when the infected cells were cultured in the presence of Ara C and labelled between 1 and 2 h p.i. Similarly, the 43 K polypeptide was also detected in cowpox virus-infected RK13 cells which were labelled between 1 and 2 h p.i. in the presence of Ara C (data not shown).

Ueda & Tagaya (1973) suggested that ECSA plays an important role in cell-mediated immunity against vaccinia virus infection. Studies on the significance of the 40 K polypeptide identified in this paper in vaccination is in progress.

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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.
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