Serologically Cross-reactive Polypeptides in Vaccinia, Cowpox and Shope Fibroma Viruses

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SUMMARY

An immunoprecipitation method coupled with SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was used to identify the serologically cross-reactive polypeptides in Orthopoxvirus (vaccinia and cowpox viruses) and Leporipoxvirus (Shope fibroma virus). Two early and four late polypeptides in cells infected with vaccinia or cowpox virus were specifically immunoprecipitated with antiserum against Shope fibroma virus. Two early and two late polypeptides in cells infected with Shope fibroma virus cross-reacted with both antiserum against vaccinia virus and antiserum against cowpox virus. The possibility of the common polypeptides being related to nucleoprotein antigen in these cross-reactive polypeptides was discussed.

On the basis of results of complement-fixation, precipitation, neutralization, fluorescent antibody and haemagglutination-inhibition tests, it is generally accepted that Poxviridae consist of several genera of serologically related viruses and also a number of agents that are serologically unrelated to other members of the Poxviridae (Downie & Dumbell, 1956; Fenner & Burnet, 1957; Mayr, 1960; Woodroffe & Fenner, 1962). However, Takahashi et al. (1959) demonstrated that there were strong cross-reactions between members of the three major genera (vaccinia, variola, fowlpox and myxoma-fibroma). Woodroffe & Fenner (1962) also investigated the serological relationships between several members of genera and proposed that the nucleoprotein (NP) antigen, which was first isolated by Smadel et al. (1942) by treating the virus particles with alkali, is an antigen common to all genera of Poxviridae. In this study, we analysed the cross-reactive antigenic components, which may contain the NP antigen, in Orthopoxvirus and Leporipoxvirus by immunoprecipitation and SDS-PAGE.

RK13 cells derived from rabbit kidney cells, purchased from Flow Laboratories, were grown in minimum essential medium (MEM; Eagle, 1959) supplemented with 10% calf serum. Vaccinia virus (Lister strain), cowpox virus (LB red strain) and Shope fibroma virus (OA strain) were used in this study. The viruses purified from infected RK13 were used as stock viruses for inoculation after titration of infectivity with plaque or focus formation. The methods for virus purification and infectivity titration were described previously (Ikuta et al. 1978a). Monolayer cells of RK13 were infected with each virus at a m.o.i. of 10 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 14C-leucine (297.0 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. 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) and centrifuged for 2 h at 100000 g and 4 °C, as described previously (Ikuta et al. 1978b). The supernatant (termed TD-lysate) was used as the soluble antigen for immunoprecipitation. Antisera were prepared as described previously (Miyamoto & Kato, 1971). All viruses for
immunization were grown on RK13 cells. Vaccinia virus (anti-vaccinia) or cowpox virus (anti-cowpox) was injected into the skin of the back of a rabbit. Two months after virus inoculation, immune serum was obtained. To prepare antiserum to Shope fibroma virus (anti-Shope), a rabbit was intracutaneously inoculated with Shope fibroma virus. The tumour grew at the site of virus inoculation and ceased to increase in size after ten days. About 1 month later, Shope fibroma virus was inoculated intravenously three times at weekly intervals. Blood was collected 10 days after the last inoculation. Indirect immunoprecipitation was conducted by the method of Kessler (1975) with slight modifications. Briefly, a 200 μl portion of TD-lysate was incubated with 5 μl of antiserum for 16 h at 4 °C and then incubated with 200 μl of 10% suspension of *Staphylococcus aureus* (Cowan I strain) in TD buffer containing 1 mg/ml of ovalbumin for 3 h at 4 °C. The mixture was centrifuged (at 2000 g for 6 min) and the immunoprecipitate was washed three times with
TD buffer and once with 10 mM-sodium phosphate buffer, pH 7.2. The immune complexes were separated from the bacterial adsorbent by heating for 3 min at 100 °C in 'sample buffer' and 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 SDS-PAGE patterns of homologously reactive immunoprecipitates and heterologously cross-reactive immunoprecipitates of vaccinia, cowpox and Shope fibroma viruses. All the TD-lysates prepared from infected cells under the conditions described above were found to contain about 30 species of antigenic polypeptides when treated with each homologous antiserum. However, no components were immunoprecipitated by normal rabbit serum or pre-immune rabbit serum against these TD-lysates, or by immune serum against the TD-lysate prepared from mock-infected RK13 cells (data not shown). As expected, the TD-lysates prepared from cells infected with vaccinia virus and from those infected with cowpox virus, which belong to the same genera of Orthopoxvirus, contained almost the same antigenic polypeptides that reacted with both anti-vaccinia and anti-cowpox sera (Fig. 1a and b). However, only in the absence of Ara C did vaccinia virus produce a polypeptide at the top of the gel which reacted with anti-vaccinia and anti-cowpox sera, but this polypeptide was not detectable in cells infected with cowpox virus. This observation agrees with the results of Gispen (1955) and Rondle & Dumbell (1962) that one major component of vaccinia soluble antigen could be demonstrated only poorly in preparations of cowpox soluble antigen. This might be explained by what is known of the LS antigen. Immunoprecipitation of the same TD-lysates with antiserum to the LS antigen showed that this polypeptide at the top of the gel did not react with anti-LS serum and the polypeptide with mol. wt. of about 100,000 is not likely to be a common component of vaccinia and cowpox viruses.

These TD-lysates were used to examine the antigenic polypeptides of Orthopoxvirus and Leporipoxvirus that cross-reacted. Three polypeptides with mol. wt. of about 65,000, 40,000 and 32,000 (65K, 40K and 32K) in the TD-lysate prepared from cells infected with vaccinia virus and in that from cells infected with cowpox virus were immunoprecipitated with anti-Shope serum (Fig. 1c). In contrast, only one polypeptide with mol. wt. of about 64,000 (64K) in the TD-lysate prepared from cells infected with Shope fibroma virus was immunoprecipitated with both anti-vaccinia and anti-cowpox sera (Fig. 1a and b, respectively). The 32K polypeptide in cells infected with vaccinia or cowpox virus was synthesized even in the presence of Ara C. The 40K polypeptide in cells infected with vaccinia or cowpox virus was also synthesized in the presence of Ara C, although the amount synthesized in cowpox virus-infected cells was considerably reduced by the presence of Ara C. The other polypeptides were synthesized only in the absence of Ara C.

We also analysed the time course of production of serologically cross-reactive polypeptides of Orthopoxvirus and Leporipoxvirus using pulse-labelled cells. For this analysis, monolayer cells of RK13 were infected or mock-infected as described above. After adsorption for 1 h, the infected cells were incubated with MEM supplemented with 2% calf serum in the presence or absence of Ara C until they were pulse-labelled. At appropriate times (see the legend to Fig. 2), they were labelled with 3 μCi/ml of 35S-methionine (442.1 Ci/mmol; New England Nuclear) in methionine-free MEM supplemented with 2% dialysed calf serum for 1 h in the presence or absence of Ara C. After pulse-labelling, TD-lysates were prepared from the cells as described above and were treated with heterologous antiserum. Then the immunoprecipitates were subjected to SDS-PAGE (Fig. 2). The TD-lysates prepared from vaccinia virus-infected cells or cowpox virus-infected cells were immunoprecipitated with anti-Shope serum, and the TD-lysate prepared from Shope fibroma virus-infected cells was immunoprecipitated with anti-cowpox serum.
For legend see opposite
The 65K and 40K polypeptides were first detectable between 3 and 4 h after infection in TD-lysates prepared from the cells infected with vaccinia virus (Fig. 2a). In TD-lysates from cowpox virus-infected cells, the 65K polypeptide was first detectable between 8 and 9 h p.i., and the 40K polypeptide was detectable between 5 and 6 h p.i. (Fig. 2b). The 65K polypeptide is not synthesized in the presence of Ara C. These results indicate that this 65K polypeptide is a virus-induced late polypeptide. On the other hand, the 40K polypeptide was synthesized even in the presence of Ara C when the infected cells were labelled between 11 and 12 h p.i. Therefore, the 40K polypeptide may be a virus-induced early polypeptide. It appears that the 40K polypeptide was too small in amount to detect during the early period after infection. Since the 32K polypeptide was already synthesized between 1 and 2 h p.i. in both vaccinia-infected cells and cowpox-infected cells labelled in the presence or absence of Ara C, this polypeptide was a virus-induced early polypeptide. The other polypeptides, which are not identified in Fig. 1, with mol. wt. of about 35000, 22000 and 14000 (35K, 22K and 14K, respectively) in the TD-lysate prepared from cells infected with vaccinia virus (Fig. 2a) and with mol. wt. of about 34000, 22000 and 14000 (34K, 22K and 14K, respectively) in the TD-lysate prepared from cells infected with cowpox virus (Fig. 2b), were also detected as cross-reactive antigenic polypeptides by pulse-labelling. This may be due to the fact that this pulse-labelling method is more sensitive than the longer labelling used in Fig. 1, or partly that the 35K and 22K polypeptides in vaccinia virus and the 34K and 22K polypeptides in cowpox virus were methionine-rich and leucine-poor polypeptides (Ikuta et al. 1978a). These three polypeptides were first detectable between 5 and 6 h p.i. in the TD-lysate prepared from vaccinia virus-infected cells (Fig. 2a) and between 8 and 9 h p.i. in TD-lysate prepared from cowpox virus-infected cells (Fig. 2b). Since none of these polypeptides was synthesized in the presence of Ara C, they were virus-induced late polypeptides.

On the other hand, in the TD-lysate prepared from cells infected with Shope fibroma virus, the 64K polypeptide was first detectable between 8 and 9 h p.i. and not detected in cells cultured in the presence of Ara C (Fig. 2c). Thus this polypeptide was a virus-induced late polypeptide. The other three polypeptides, which were not detectable in Fig. 1, with mol. wt. of about 46000, 43000 and 41500 (46K, 43K and 41.5K, respectively) were detected by this pulse-labelling method (Fig. 2c). Since the 41.5K polypeptide was first detectable between 8 and 9 h p.i. and was not synthesized in the presence of Ara C, it was also a virus-induced late polypeptide. The other two polypeptides (46K and 43K) were detectable in infected cells labelled between 1 and 2 h p.i. in the absence, or even in the presence of Ara C and thus these polypeptides were virus-induced early polypeptides.

Thus, six polypeptides and four polypeptides were detected, respectively as the cross-reactive antigenic components in cells infected with Orthopoxvirus and Leporipoxvirus. Vaccinia virus-infected cells have two early (40K and 32K) and four late (65K, 35K, 22K and 14K) cross-reactive polypeptides. Cowpox virus-infected cells have two early (40K and 32K) and four late (65K, 34K, 22K and 14K) cross-reactive polypeptides, while Shope

Fig. 2. Time course of production of virus-induced antigenic polypeptides reacting with heterologous antiserum. RK13 cells were infected with vaccinia virus, cowpox virus, or Shope fibroma virus or were mock-infected in the presence (+) or absence (−) of Ara C (50 μg/ml). Infected (I) or mock-infected (M) cells were incubated with MEM supplemented with 2% calf serum in the presence or absence of Ara C until pulse-labelled and then labelled with 35S-methionine in methionine-free MEM supplemented with 2% dialysed calf serum for 1 h at the times indicated in the figure (h p.i.) in the presence or absence of Ara C. The TD-lysates prepared from vaccinia and cowpox virus-infected cells were treated with anti-Shope serum and the TD-lysates prepared from Shope fibroma virus-infected cells were treated with anti-cowpox serum. The immunoprecipitates were analysed by SDS-PAGE and then fluorography. Mol. wt. of polypeptides were calculated as described in the legend to Fig. 1.
fibroma virus-infected cells have two early (46K and 43K) and two late (64K and 41.5K) cross-reactive polypeptides. However, no equivalently sized polypeptide was immunoprecipitated between the cells infected with Orthopoxvirus and those with Leporipoxvirus. In general, it can be inferred that polypeptides of the same mol. wt. in preparations of these two genera might be antigenically unrelated and antigenic determinants of shared components do not necessarily appear as polypeptides of the same size. For example, anti-Shope serum immunoprecipitated a 40K polypeptide from cells infected with vaccinia virus and with cowpox virus, as well as from cells infected with Shope fibroma virus (Fig. 1c). But no equivalent sized polypeptide was immunoprecipitated from Shope fibroma virus-infected cells by anti-vaccinia or anti-cowpox sera (Fig. 1a), although these antisera immunoprecipitated from cells infected with vaccinia or cowpox virus. These observations imply that the 40K polypeptide in Shope fibroma virus-infected cells is not cross-reactive with the 40K polypeptide in cells infected with vaccinia or cowpox virus. Also, there are two reasons why other cross-reactive components unresolved here could be present. First, about 30% of the trichloroacetic acid-insoluble radioactivity of the infected cells could not be solubilized with TD buffer. Therefore, we can analyse no more than 70% of the infected cells and the antigenic polypeptides in the other 30% in the precipitate may be unresolved. Second, the antigenic determinants of some polypeptides may be removed by detergent in TD buffer. Thus, we cannot determine at present which of the polypeptides in Orthopoxvirus-infected cells are cross-reactive with those in Leporipoxvirus-infected cells.

The relationship between these cross-reactive polypeptides and the NP antigen is unknown. The NP antigen, which constitutes 50% of the mass of the virus particles and contains all the virus DNA (Smadel et al. 1942), is associated with the internal protein, as shown by the immunoadsorption method (Woodroofe & Fenner, 1962). We previously analysed the virion structural polypeptides of vaccinia, cowpox and Shope fibroma viruses (Ikuta et al. 1978a). Almost all the components identified in this study, except the 40K and 32K polypeptides in vaccinia virus-infected cells and 32K polypeptide in cowpox virus-infected cells, were constituents of the virions, judging from their mobilities on SDS-PAGE. The 65K polypeptide was one of the major structural polypeptides of vaccinia virions and may correspond to one of the polypeptides of VP4 shown by Sarov & Joklik (1972), who demonstrated that the VP4 was a major internal protein of vaccinia virions. These results suggest that the 65K polypeptide in vaccinia virus is one of the NP antigens. Although no information for the internal proteins of cowpox virions or Shope fibroma virions has yet been obtained, the pattern and mol. wt. of the VP4 of vaccinia virions are similar to those of cowpox virions or Shope fibroma virions (Ikuta et al. 1978a), suggesting that the 65K and 64K polypeptides are respectively also among the NP antigens in cowpox virus and in Shope fibroma virus.

For identification of the NP antigen, studies are required to determine which of the cross-reactive antigenic polypeptides identified in this study is located in the NP fraction prepared from virus particles.

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