Crossed Immunoelectrophoretic Characterization of Virus-Specified Antigens in Cells Infected with African Swine Fever (ASF) Virus

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SUMMARY

Crossed immunoelectrophoresis was applied for the characterization of five specific antigens in African swine fever (ASF) virus infected cells. Monospecific antisera were prepared against three individual antigens in the precipitation arcs.

Relatively little work has been performed on the antigenic structure of African swine fever (ASF) virus. It is known, however, that precipitating antibodies against some virus-induced products are formed in the pig following natural infection with ASF virus (Coggins & Heuschele, 1966; Stone, DeLay & Sharman, 1968). These antibodies form the basis of immunofluorescence and immunoelectro-osmophoresis diagnostic tests (Pan et al. 1974). Crossed immunoelectrophoresis has mainly been applied to the study of serum proteins (Axelsen, Kröll & Weeke, 1973), and recent work has shown the successful application of the technique to the study of virus antigens (Faber Vestergaard, 1973; Dalsgaard, 1976). We have followed a procedure of antigen preparation designed to investigate such virus-specified antigens which can be released from infected cells by treatment with the non-ionic detergent Triton X-100.

The monkey kidney cell line (MS) was grown in Roux bottles. When the monolayer was confluent the bottles were infected with ASF virus at a high multiplicity (10/1). Twenty-four h after infection the cells were harvested. At this time no major cytopathic effect was observed, except for certain changes in cell shape. The cells were scraped off the glass with a rubber policeman and pelleted by centrifugation at low speed. The supernatant fluid was discarded and the cells were resuspended in 0.01 M-tris/HCl, pH 7.5, containing 10 % Triton X-100 (scintillation grade, Serva, Heidelberg). Three parts of buffer were added to one part of packed cells. The mixture was sonicated in an ice bath for 3 × 30 s at maximum output (MSE type PG 100). The resulting milky suspension was left overnight at 4 °C and the sonication procedure was repeated after 18 h. The mixture was then ultracentrifuged at 100000 g for 1 h at 4 °C, and the clear layer between an upper lipid layer and a bottom precipitate was isolated by puncturing the centrifuge tube with a syringe. This layer was kept at −80 °C and served as the antigen for crossed immunoelectrophoresis. Uninfected cells were treated in the same way and served as control antigen.

The antiserum used for electrophoresis was a porcine hyperimmune serum prepared by repeated experimental infection with ASF virus infected blood. A normal porcine SPF serum served as a control. Crossed electrophoresis was carried out essentially as described by Faber Vestergaard (1973). Four first dimensions were run on a 9 × 11 cm plate. The second dimensions were run on 10 × 10 cm plates. One per cent agarose was used for the gels and 1 % Triton X-100 was included in the gels and buffer vessels to avoid any reaggregation of sample material. Fifteen μl of antigen preparation (28 μg N using the method of Lowry et al. 1951) was electrophoresed in the first dimension gel for 2 h at 10 v/cm and 200 μl of antiserum was applied in the second dimension gel. The second run was carried...
out at 1.5 V/cm overnight. The plates were washed, pressed, dried and stained with Coomassie brilliant blue as described (Axelsen et al. 1973). By running control antigen against ASF virus antiserum, and ASF virus antigen against control serum, it could be established that non-specific precipitation of host antigens did not take place. In Fig. 1 the immunoelectrophoretic pattern of ASF virus antigen against ASF virus hyperimmune serum is shown. Five distinct precipitates could be demonstrated. Using concanavalin A in an intermediate gel (Bøg-Hansen, 1973) it could be demonstrated that antigen number V was glycosylated (results not shown). The above-mentioned antigen preparation was re-tested after a period of six months, and, using two different hyperimmune ASF virus antisera, no difference could be seen in the resulting crossed immunoelectrophoretic pattern. In another antigen preparation, 10% of Trasylol solution (23000 KIU/ml; Bayer) was included at the time of re-suspension of packed infected cells. Trasylol (aprotinin, WHO proposed name), a broad range protease inhibitor (Trautschold, Werle & Zick-Rüdel, 1966), is a basic polypeptide isolated from bovine pancreas. This substance was added to the antigen preparation to investigate whether inhibition of proteolytic activity might alter the concentration of individual antigens. Trasylol had no effect on antigens I to IV, but it significantly increased the yield of the glycoprotein antigen V. This was estimated by crossed immunoelectrophoresis, which is a quantitative technique, the area enclosed by the precipitation arc being directly proportional to the antigen/antibody ratio of the system. The increase of antigen V never exceeded a factor of 2.5.

Fig. 1. Crossed immunoelectrophoresis: 15 μl ASF antigen against 200 μl ASF hyperimmune porcine serum. Anode was to the left in the first dimension and at the top in the second dimension. Staining was by Coomassie brilliant blue.
Since crossed electrophoresis is carried out under the application of electric current in the second dimension, all non-precipitated constituents of the antigen mixture are usually carried to the buffer vessels. This situation opens the possibility of preparing monospecific antisera against individual antigens present in the precipitation arcs, and this was investigated using ASF virus antigens I to V. Five groups of five pigs were each immunized with three precipitation lines cut from unstained crossed-immunoelectrophoretic plates and emulsified in Freund's incomplete adjuvant. The precipitation lines were easily cut from the agarose plate without contamination from neighbouring lines because of the excellent resolving power of the crossed electrophoresis. The pigs were boosted at intervals of 2 weeks until precipitating antibodies could be detected. Using this method it was possible to produce monospecific sera against antigens I and IV, and against the glycoprotein antigen V.

In Fig. 2, 15 µl (36 µg N) of ASF virus antigen preparation (prepared with Trasylol) was electrophoresed against 0.5 ml of individual monospecific antisera. No reaction from host cell material was seen and the sera only reacted with one of the individual antigens. To reduce the background staining the lipoproteins were removed from the monospecific sera by centrifuging at 100000 g for 1 h. It was not possible to obtain an immune response against antigens II and III. They may be either weak immunogens or the quantity available may be

![Fig. 2. Same procedure as Fig. 1 with 15 µl ASF antigen but with 0.5 ml monospecific porcine serum, prepared against antigens I, IV and V respectively.](image-url)
Short communications

too small. One must remember that the antigenic mass in such precipitation lines is very low, usually less than 1 µg of protein. As demonstrated, however, such minute quantities are in many cases sufficient to induce an immune response.

Crossed immunoelectrophoretic characterization of herpes hominis virus antigens revealed the presence of 11 specific antigens (Faber Vestergaard, 1973) under conditions similar to the experiments of the present paper. The molecular weights of herpes hominis virus DNA (Becker, Dym & Sarov, 1968) and of ASF virus DNA (Enjuanes, Carrascosa & Vifuela, 1976) have been reported to be 10^8. On this basis we had expected more than five specific antigens to be expressed in our system. This finding however, is in good agreement with a recent report (Black & Brown, 1976) on the structural proteins of ASF virus which demonstrated only five structural proteins, whereas in herpes hominis virus at least 12 major structural proteins have been reported (Honess & Roizman, 1973).

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