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ATTEMPTS AT DETECTION OF ACTOMYOSIN ASSOCIATED WITH INFLUENZA VIRUS*

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Summary. — Influenza virus strains A/Scotland/74, A/Hong Kong /68, A/Port Chalmers/73 and the MRC-12 recombinant were tested with immune antiserum against actomyosin. As shown by electron microscopy, the serum aggregated virus particles, but only after bromelain treatment (without haemagglutinin and neuraminidase spikes). In rocket electrophoresis the serum gave positive precipitation reaction with all the strains tested, and with virus from various hosts (chick embryo, monkey kidney cell culture, mice after adaptation). Therefore the host protein presumably is present in the influenza virus structure irrespective of the strain or the host in which the virus is grown.

Key words: influenza virus; host components

Introduction

As reported previously, the formation of smooth-muscle antibody type autoantibodies is elicited by natural influenza infection (Łoza-Tulimowska *et al.*, 1976), immunization of humans with inactivated vaccine (Łoza-Tulimowska *et al.*, 1979), immunization of animals with purified influenza virus (Łoza-Tulimowska *et al.*, 1977).

In the present study we attempted to show whether purified influenza virus reacts with immune antiserum against actomyosin.

Materials and Methods

Actomyosin antisera. An actomyosin preparation from the smooth muscle layer of hen's gut was obtained by courtesy of Dr. Z. Górecka, Dept. of Biochemistry, Institute of Experimental Biology, Polish Academy of Sciences, Warsaw. Rabbits weighing 3 kg were immunized intramuscularly with actomyosin + complete Freund's adjuvant. The sera obtained were tested by indirect immunofluorescence (Łoza-Tulimowska *et al.*, 1977) for actomyosin antibodies, whose titre was 320. Some experiments (rocket electrophoresis) were performed with a rabbit serum which showed a "natural" autoantibody titre of 1280.

Viruses. The following influenza virus strains grown in the allantoic cavities of embryonated hen's eggs were used: A/Scotland/74(H3N2), A/Hong Kong/68(H3N2), A/Port Chalmers/73(H3N2)

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and MRC-12(H3N2) recombinant. The strain A/Scotland/74 was also propagated in primary monkey kidney cell cultures, and in the lungs of mice after adaptation to this host. All virus harvests were concentrated by ultracentrifugation and purified by sucrose density gradient centrifugation, to obtain preparations with virus protein contents of about 15 mg/ml.

Electron microscopy. A/Scotland/74 virus was used. A part of the virus preparation was treated with bromelain (Brand and Skehel, 1972) to obtain particles without haemagglutinin and neuraminidase spikes. Untreated and bromelain-treated virus suspensions in volumes of 0.1 ml were incubated with 0.9 ml of the globulin fraction of rabbit actomyosin antiserum for 1 hr at 37 °C. The mixtures were then centrifuged under refrigeration for 40 min at 14000 × g. The sediment was resuspended in 3 ml of twice distilled water, vigorously shaken, and centrifuged. The final sediment was suspended in 0.1 ml of twice distilled water and dropped on formvar-coated 100-mesh copper grids. The preparations were negatively stained with 1% sodium phosphotungstate. In controls, actomyosin antibodies were replaced by a) phosphate buffered saline (PBS), b) normal rabbit serum, and c) immune antiserum homologous for the virus used.

Rocket electrophoresis was performed according to Oxford and Schild (1977). Plates (6 × 9 cm) were coated with 1% agarose (9.5 ml) containing the globulin fraction of actomyosin rabbit antiserum (0.5 ml). Purified virus preparations of the strains tested (see above) were treated with sodium dodecylsulphate (SDS) for 30 min at room temperature, and distributed in 2.5 µl portions into grooves of the electrophoresis plates. Electrophoresis lasted for 1 hr at 8 V/cm. In controls, actomyosin antibodies were replaced by normal rabbit serum and with antibodies against A/Scotland/74 virus. In experiments on A/Scotland virus originating from various hosts, virus preparations for electrophoresis were also treated for 2 min at 100 °C with Norrby's reagent.

Results

Electron microscopy showed untreated virus particles to aggregate when incubated with virus antiserum, but not with actomyosin antiserum. Aggregation was observed when actomyosin antiserum was incubated with bromelain treated virus. All controls were negative.

Interaction of SDS-treated virus with actomyosin antibodies was demonstrated by rocket electrophoresis. All the strains tested gave a positive precipitation reaction with virus antiserum (Fig. 1) and with actomyosin antiserum (Fig. 2). Rockets were not found with SDS-untreated virus antigens and in controls.

Figs 3 and 4 illustrate the reaction of actomyosin antibodies (serum from a rabbit with a high titre of "natural" antibodies, and serum from an immunized rabbit) with viruses from various hosts. The reaction was positive irrespective of the method of antigen treatment (SDS or Norrby's reagent). Intensity of the reaction was the highest with virus from chick embryos and the lowest with virus from cell culture, in spite of that virus protein contents in the preparations were the same.

Discussion

The present observations represent a further step in the search for host components incorporated into the influenza virus structure, and the site in the virion where they may occur.

When incorporated into the virion, actomyosin may induce autoantibody formation (Łoza-Tulimowska *et al.*, 1976, 1977, 1979) with all pathogenetic implications of this fact. On the other hand, it could be an element of virus variability, if actomyosins from various species differ in immunological specificity.

An association of actin (one of actomyosin constituents) with the virion has been suggested for some viruses, e.g. paramyxoviruses and mouse mammary tumour virus (Damsky *et al.*, 1976, 1977; Wang *et al.*, 1976). It has been postulated that actin is actively incorporated into the virion.

The present results showed that the influenza virus particle contains the actomyosin antigen. The reaction of actomyosin antiserum was positive only with bromelain-treated or detergent-disrupted virus. It may be suggested, therefore, that the antigen is not associated with surface antigens, but rather with the internal virion structure. The presence of actomyosin may be considered a feature depending neither on the strain nor host in which the virus is grown.

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Explanation of Figures (Plates XXXVIII and XXXIX):

Fig. 1. Rocket electrophoresis with A/Scotland virus antiserum. Antigens from left to right: A/Scotland, MRC-12, A/Hong Kong, A/Port Chalmers.

Fig. 2. Rocket electrophoresis with actomyosin antibodies. Antigens as in Fig. 1.

Fig. 3. Rocket electrophoresis with rabbit serum containing "natural" actomyosin antibodies. Antigens from left to right: A/Scotland/74 virus from chick embryos, mouse, monkey kidney cells treated with SDS: from chick embryos, mouse, monkey kidney cells treated with Norrby's reagent.

Fig. 4. Rocket electrophoresis with serum of actomyosin-immunized rabbit. Antigens as in Fig. 3.