Cell Fusion by the Antigen-antibody Complex of Sendai Virus Studied by Electron Microscopy

(Accepted 19 April 1974)

SUMMARY

Cell fusion of Ehrlich ascites tumour cells caused by antigen-antibody complexes of Sendai virus was studied by electron microscopy. The fusion process was similar to that produced by virus. The fusion of envelopes of virus particles aggregated by antibodies and cell membranes was not observed under the conditions employed, though cell fusion was enhanced. This finding supported the hypothesis that the formation of cell-cell bridges but not of cell-envelope-cell bridges is involved in the initiation of cell fusion.

In 1968, we studied fusion of Ehrlich ascites tumour cells by Sendai virus particles \textit{in vitro} by electron microscopy and suggested that degradation of cell membranes by adsorbed virus and the subsequent unity of adjacent cells through the breaks led to the formation of a cytoplasmic bridge (Hosaka & Koshi, 1968). Then, Morgan & Howe (1968) and Howe & Morgan (1969) demonstrated the fusion of virus envelopes and cell membranes during haemolysis or entry by Sendai virus. Apostolov & Almeida (1972) and Apostolov & Poste (1972) proposed the hypothesis of cell-envelope-cell bridge formation for cell fusion from their findings of envelope cell fusion by Sendai virus. In this report, we present an additional observation supporting the formation of cell-cell bridges in Sendai virus-induced cell fusion, using the system of Ehrlich ascites tumour cells-virus-antibody complex of Sendai virus (Okada, Yamada & Tadokoro, 1964), in which cell fusion was enhanced and the fusion between virus envelopes and cell membranes was expected to be inhibited, though it was only concerned with fusion from without. Recently, Bächli, Auget & Howe (1973) reported results supporting the cell-cell bridge formation in Sendai virus-induced cell fusion, though they discounted the direct participation of Sendai virus particles in the fusion site.

Sendai virus (HVJ), Z strain, was partially purified by differential sedimentation and the haemagglutinating (HA), haemolytic and cell fusion activities were measured as described previously (Hosaka, Kitano & Ikeguchi, 1966). Vol. of 6 ml of partially purified virus particles (12,000 H.A.U./ml) were mixed with 3 ml of twofold serial dilutions of anti-Sendai rabbit serum, which was prepared by injection of disrupted virus particles (Hosaka, 1968). The mixtures were incubated at 37 °C for 1 h and then kept overnight in the cold. The mixtures were centrifuged at 6000 rev/min for 30 min and the precipitates resuspended with vigorous stirring in 6 ml of Hanks’ solution. The HA, haemolytic and fusion activities of these antigen-antibody complexes are shown in Fig. 1. The HA activity was greatly reduced and the haemolytic activity was reduced with a shoulder but the fusion activity of the complexes formed with 2^{-5} and 2^{-6} dilutions of antiserum was enhanced. These results are essentially similar to those originally reported by Okada \textit{et al.} (1964). An infectivity test showed that the complexes had EID_{50}/HA ratio of about 10^6, which was the same as that of untreated virus particles.

0.75 ml of antigen-antibody complex, prepared with 2^{-5} antiserum dilution, was mixed with an equal vol. of 10 \% suspension of Ehrlich ascites tumour cells in Hanks’ solution.
Fig. 1. HA (■—■), haemolytic (○—○) and fusion (▲—▲) activities of the antigen-antibody complexes of Sendai virus formed with twofold serial dilutions of anti-Sendai serum. The complexes were sedimented by sedimentation at 6000 rev/min for 20 min, resuspended and assayed. Haemolysis (HL) and fusion were measured with 0.05 ml and 0.75 ml samples of the resuspended complexes, respectively. The control with no serum was directly assayed without sedimentation. The fusion index was calculated according to the following formula (Okada & Tadokoro, 1962):

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\text{Fusion index} = \frac{\text{cell number in non-virus control tube}}{\text{cell number in test tube}} - 1.0
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with 2 % calf serum kept at 4 °C for 10 min, and then incubated at 37 °C. Samples were removed at appropriate intervals for electron microscopy. The method of electron microscopy was the same as described previously (Hosaka & Koshi, 1968).

Fig. 2 shows the adsorption of complexes to cell membranes in the cold. Free surfaces of the complexes were covered with irregular structures like turf, which were probably made of antibodies attaching to the spikes. Some of them appeared to be loops (in the inset of Fig. 2). Possible antibodies bridging two virus particles were also observed. These modes of attachment of antibodies to virus particles were similar to those seen with influenza virus (Lafferty & Obertelis, 1963). One complex had a larger contact area with the cell membrane than free virus particles, as shown in Fig. 2, where the cell membranes were partly invaginated. The complexes appeared to adsorb to cell membranes by unreacted intact surface, because the contact zones formed between cell membranes and virus envelopes had the same width of 15 nm as in the adsorption of untreated virus particles (Hosaka & Koshi, 1968). In the contact zones, an intermediate line (indicated by i in Fig. 2) was frequently observed; this could be due to the antibodies attached to the spikes of 10 nm and squeezed in the narrow contact zone.

Loosely packed nucleocapsids with a rough surface were seen within some large virus particles (Fig. 2). This appearance may have been caused by antibodies which had penetrated the broken envelopes. No cell fusion was observed in the cold (at 4 °C).

After 1 min incubation at 37 °C, cytoplasmic bridges (Fig. 3) as well as close contacts (Fig. 4) of adjacent cell membranes were observed frequently on or near small complexes. At this time many more bridges were observed than in the fusion by untreated virus particles (Hosaka & Koshi, 1968), so bridge formation may have proceeded more rapidly.

After 2 min, many enlarged cytoplasmic bridges were observed. At 5 min, intercellular cavities containing a large complex were seen (Fig. 5). The virus aggregates seen in vesicles (Fig. 5 and 6) may have been trapped in an intercellular cavity and then enclosed by the
Fig. 2. Adsorption of antigen-antibody complexes to Ehrlich ascites tumour cells in the cold. In the contact zones of about 15 nm between the virus envelopes and cell membranes, an intermediate line (i) is seen. Typical forms of loop and bridge of antibody attachment were indicated by arrows l and b, respectively. A typical nucleocapsid with a rough surface was indicated by an arrow n.

cell membrane. These vesicles were irregular in shape and often completely filled with virus particles (Fig. 6). In cells infected by non-aggregated virus particles (Hosaka & Koshi, 1968), irregular vesicles containing a number of virus particles were not seen. Not only that, large vesicles with several virus particles usually had a circular outline and may have
resulted from fusion of small vesicles containing one virus particle each. Throughout the period of incubation we observed neither fusion of envelopes of aggregated virus particles and cell membranes nor released nucleocapsids in the cytoplasmic bridges. This finding did not necessarily contraindicate a small number of envelope-cell membrane fusions that we could not easily detect in the present system, because the complexes still had an EID_{60}/HA ratio of about 10^6. If penetration was mediated by envelope fusion, the envelope fusion might well occur in a very small number of cells. The envelope site of an occasional virus particle trapped in a complex and not combined with antibody might fuse with the cell membrane, which results in entrance of nucleocapsids into cytoplasm. However, there would be a rare chance that such fusion happens in plural sites, to form antibody-attached envelope bridge for cell fusion.

Bachi et al. (1973) showed that the cytoplasmic bridges of human erythrocytes were induced with Sendai virus within 30 s incubation at 37 °C and the bridges were not associated with virus antigen, which is consistent with the cell-cell bridge hypothesis. They considered that the displacement of the glycoprotein particles of cell membranes induced by Sendai virus particles might be the fundamental process leading to fusion or lysis.

Recently, Hosaka (1974), Homma & Okuchi (1973) and Scheid & Choppin (1974) presented findings that the smaller one of two glycoproteins of active Sendai virus may be concerned with virus haemolysis or fusion, while the larger one may be concerned with HA and neuraminidase activities (Tozawa, Watanabe & Ishida, 1973; Scheid & Choppin, 1974). The direct interaction of the virus glycoprotein and cell membranes seemed to be necessary for fusion.

The antigen-antibody complexes of Sendai virus particles had larger areas of contact with cell membranes per complex than free virus particles and possibly they mediated many and sufficiently strong cell contacts to assure the formation of cytoplasmic bridges, which could be responsible for the enhancement of cell fusion. This is consistent with the interpretation proposed by Okada et al. (1964) and Okada & Murayama (1968).

We interpret the relationship between cell fusion and envelope fusion in the following way. The smaller glycoprotein of Sendai virus interacts with cell membranes and brings about changes in the membrane on the site of virus adsorption. The contiguous sites of altered membrane may interact to form a cytoplasmic bridge for cell fusion. Alterations may also occur in the envelope, resulting from interaction with cell membranes. If an altered area of virus envelope happens to be contiguous with altered cell membrane then envelope-membrane fusion could occur.

We wish to thank Professor K. Fukai for his support and interest and Professor Y. Okada for his advices.

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Fig. 3. A cytoplasmic bridge (cb) seen next to a complex (C). 1 min incubation at 37 °C.
Fig. 4. Close contact of adjacent cell membranes, seen near a complex (C), indicated by an arrow. 1 min incubation at 37 °C.
Fig. 5. A large complex (C) in an intercellular cavity formed among enlarged bridges. 5 min incubation at 37 °C.
Fig. 6. An irregular shaped vesicle (ves) tightly enclosing a virus particle aggregate of the complex. 20 min incubation at 37 °C.

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REFERENCES


(Received 26 February 1974)