Studies on Sendai Virus Cell Fusion Factor* (33441)

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The use of Sendai virus induced cell fusion to study various problems of cell biology has received considerable attention in the past 3 years. The technique has proven useful for studying nuclear-cytoplasmic relationships (1, 2) and has also permitted recovery of "latent" viral genomes (3, 4), viral infection of normally resistant cells (5) and studies on the production and mechanism of interferon action (6, 7). During the course of work on cell fusion in our laboratory, we studied several technical aspects that proved important in order to achieve a high proportion of heterokaryons necessary for some experiments with interferon.

The basic approach to Sendai virus induced cell fusion is that first published by Okada (8) and later modified and applied to interspecific cell fusion by Harris and coworkers (1). The technique outlined by Harris has been used, apparently without modification, by other workers. We have found that variations in two separate steps of this technique caused significantly different results in the efficiency of fusion. Since these variations have not been discussed in detail in previous publications, we are reporting them so that other investigators who require systems with high percentages of heterokaryons may utilize our data in their cell fusion work. Our studies also add further information about the nature of the cell fusion factor (CFF).

Materials and Methods. Sendai virus. Our strain of Sendai virus was obtained from Dr. Samuel Baron of the National Institute of Allergy and Infectious Diseases, National Institutes of Health where it had been carried for many passages in embryonated chick eggs. In our laboratory the virus has undergone a number of additional passages as follows: Stock virus of high infectivity ($10^{8.5}TCID_{50}$ per ml) was diluted to 10^{-3} with Eagles diploid growth medium (DGM). Two-tenths milliliter of this suspension was inoculated into the allantoic sac of 10–11-day chick embryos. The eggs were incubated at 36.5° for 36–40 hr, kept at 4° overnight, and the allantoic-amniotic fluid (AF) was collected sterilely. The AF was then clarified by centrifugation at 1000*g* for 10 min at 4°. This crude AF (Hemagglutin (HA) titer 1000–4000 HA units/ml) was either used directly or a further "concentration" step performed by centrifuging at 32,000*g* for 30 min. The resulting pellet was resuspended in DGM with 10% fetal bovine serum (FBS) and stored in aliquots at —70°. Infectivity assays were performed by microhemadsorption on roller tubes of African green monkey kidney cells (obtained from Microbiological Assoc.). Hemagglutinin was titrated with fresh guinea pig red cells.

Cells. HeLa cells, clone S-3, were obtained from the National Institutes of Health media section. Immediately prior to fusion, cells were trypsinized, washed 2 times, counted and diluted to 5×10^6 cells/ml. AH-1 cells, a continuous line of normal human fibroblasts, were also obtained from the National Institutes of Health media section and prepared for fusion by trypsinization. Chick red blood cells (RBC) were obtained by exsanguinating 15–18-day-chick embryos. All buffy coat cells were removed by repeated centrifugation and removal of the top layer of cells. Careful examination of smears of the final red cell suspension revealed only RBC's.

UV inactivation of Sendai virus was achieved by placing 2.0 ml of virus suspension in a 60-mm plastic petri dish. The UV source was a long wave mineral light whose output was calibrated with a Laterjet dosimeter. The petri dish was placed at a distance from the UV source at which 1500

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ergs/cm^2/sec was emitted. The solution was mixed by gentle agitation and intermittent pipetting during exposure.

Cell fusion. The cells to be fused were placed in a glass tube (15 ml capacity), centrifuged at 500*g* for 5 min and the cell pellet then was resuspended in 1 ml of the virus suspension. The concentration of cells was approximately 10^7/ml except that when chick erythrocytes were being fused they were present in 100-fold excess of the human cell (i.e., 10^9 RBC and 10^7 AH-1 cells). The cell–virus mixture was placed in ice for 20–30 min and then transferred to a 37° water bath for 90–120 min with intermittent mixing. The cells were then centrifuged to a pellet, the supernatent was discarded, and the pellet was resuspended in DGM with 10% FBS. The cells were then plated out in petri dishes containing cover slips and incubated at 37° in an atmosphere of 5% CO_2.

Assay for cell fusion factor. Cover slips of the fused cells were removed from the growth medium 24–48 hr after fusion, rinsed well to dislodge nonadhering cells including most of the nonfused RBC's, placed in formol–sublimate fixative for 1–2 hr and then stained with hematoxylin and eosin. Random fields were scored by direct microscopic examination. Control slides of cell mixtures, treated as above but not exposed to Sendai virus, were always examined to rule out spontaneously occurring multinucleate cells. If these were present (as occurred significantly in the HeLa cells) the level of "background fusion" was subtracted from any experimental score. Various methods of indicating fusion can be used, e.g., (i) number of nuclei/cell; (ii) percentage of cells that are heterokaryons; and (iii) ratio of nucleus A to nucleus B; etc. Each scoring method conveys slightly different information and the method to be used depends on the needs of a particular experiment. For instance, method (iii) above was the most sensitive indicator of the fusing ability of the virus in the AH-1-RBC system, but for experiments testing the action of interferon on these heterokaryons, method (ii) was the most important criterion.

Findings. In our experiments which utilized virus induced cell fusions in a number of different cell systems, we noticed two phenomena that significantly affected the degree of fusion. One important variable was the amount of UV irradiation to which the virus was exposed; the second observation was the unexpected finding that crude allantoic fluid was much more effective than the concentrated virus. Experiments were then done to further define the effects of these two variables.

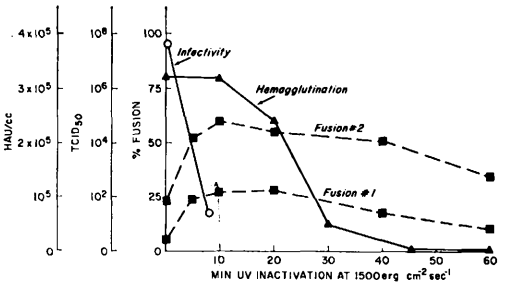

FIG. 1. Effect of UV on Sendai virus activities: (○), viral infectivity ($TCID_{50}$); (▲), hemagglutination of fresh guinea pig cells (■), 2 separate experiments in which HeLa cell fusion was assayed by direct microscopy; 400 cells/point were counted and only those with 3 or more nuclei per cell were considered fused.

Effect of UV on the cell fusion factor. Most cell fusion systems described in the literature have called for "10 min UV inactivation of the virus." Only a few reports have specified the dose. The obvious reason for exposing the virus to UV is to destroy viral infectivity to avoid the problem of viral growth and CPE. Sendai virus infectivity is, indeed, quite sensitive to UV as shown in Fig. 1. However, we found the UV dose can also alter the ability of the virus to induce cell fusion. Figure 1 shows the effect of UV on the infectivity and hemagglutinating ability of Sendai virus and 2 separate experiments in which the effect of UV on the CFF was assayed in HeLa cells. The HeLa cell system is one in which cell fusion is easily obtained and the effect of the UV dose could, therefore, be accurately evaluated. Both fusion experiments demonstrate that there is a UV dose (10–15 min at 1500 ergs/cm^2/sec) at which the CFF is maximal in this system. Nonirradiated virus is clearly less active. This phenomenon is also true in other cell fusion systems (e.g., see no. 8 of Fig. 2). In

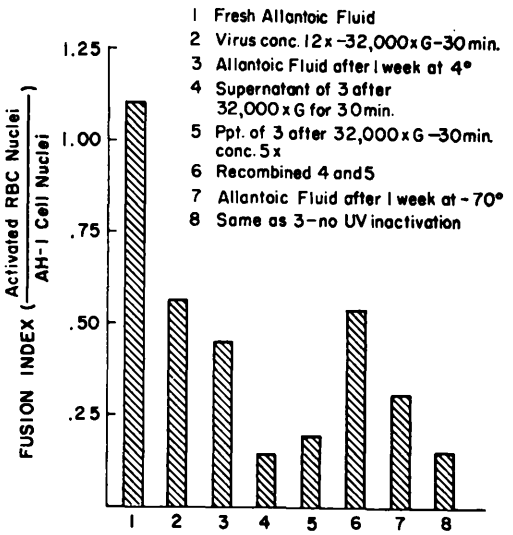

FIG. 2. Lability of Sendai virus cell fusion factor: The fusion index is the ratio of fused erythrocyte nuclei to the human (AH-1) nuclei; at high levels of fusion (index >.75) there were commonly two or more of each type of nucleus within one multinucleate cell. Preparations 1–7 were UV inactivated for 10 min.

addition Fig. 1 demonstrates the nonidentity of the CFF with the hemagglutinin of Sendai virus.

Effects of virus concentration and storage on the cell fusion factor. In some cell fusion systems, in contrast to the HeLa cell system used above, high levels of cell fusion are vary difficult to obtain. Harris originally commented on the variable ease of fusion of different cells (1). Our experiences with a number of different cell systems has confirmed that, in general, large epithelial cells (e.g., Hep-2, HeLa) fuse readily while small round cells with scanty cytoplasm (e.g., lymphoid cells, RBC's) fuse with great difficulty. During our attempts to achieve high degrees of fusion (>95% heterokaryons) in the AH-1-chick RBC system we found that the CFF is labile and is partially lost when the virus is concentrated by centrifugation as suggested by Harris (1). Figure 2 shows the results of a fusion between AH-1 cells and chick erythrocytes in which the only variable was the Sendai virus used. When fresh (within a few hours of harvesting) allantoic fluid was used (1000 HA units/ml) a high degree of fusion was achieved in which >95% of the cells were heterokaryons, some with multiple activated RBC nuclei. If the same AF was centrifuged at 32,000*g* for 30 min and the virus concentrated to 12,000 HA units there was a much lower incidence fusion of the erythrocyte nuclei. As indicated by test systems 4, 5, and 6, the CFF resides neither wholly in the precipitated virus nor in the supernatant—but can be reconstituted by combining the two fractions. The lability of the CFF is shown by the observation that its effectiveness is reduced by more than 50% when stored for 1 week at either 4 or —70°.

Discussion. The above findings indicate that some heretofore accepted aspects of virus-induced cell fusion techniques must be carefully controlled if one wishes to work with a cell system in which fusion is difficult to attain. The dose of UV irradiation used affects the CFF, but not in a simple linear relationship. The dose-activity curve actually found suggests that cell fusion may depend on several viral factors and that some "fusion inhibiting component" is more sensitive to UV than is the CFF per se.

The CFF of Sendai virus is clearly not directly related to either the infective virion or to the hemagglutinin—since both UV and centrifugation have divergent effects on these three activities. Our observations on the lability of the CFF add further support to this finding since viral infectivity and hemagglutination ability remain constant during many weeks of storage at —70° while the CFF is partially lost. Okada came to similar conclusions in his original studies on this virus (8). Work done by Kohn (9) on Newcastle disease virus and by Harter and Choppin (10) on Visna virus also showed divergence of these factors from cell fusion ability. One difference was observed with Visna where the fusion factor did appear to be associated with the virion itself, as shown by identical behavior during high speed centrifugation. Our finding that maximally active CFF was associated with neither the virus particle nor with the supernatent but could be regained by combining these two fractions suggests that this phenomenon of cell fusion may actually result from several viral components.

The simple experiments reported here leave unanswered many questions regarding the nature of the Sendai virus CFF. However, to solve the practical problem of achieving a high proportion of heterokaryons in systems where cell fusion occurs with difficulty, we suggest using freshly harvested allantoic fluid and avoiding any concentration of virus since this seems to have the opposite effect on the CFF. Careful control of the UV dose is also mandatory. Moreover, our experience has convinced us that each different mixture of cells that one wishes to use for fusion must be approached as a new experiment for which optimal conditions (cell concentrations, proportions, virus dose) must be found.

Summary. Induction of high levels of cell fusion by UV inactivated Sendai virus depends both on the virus preparation and the types of cells being fused. Studies on the optimal UV dose reveal a biphasic effect on the viral cell fusion factor (CFF). Other studies show that the CFF is very labile, is separate from both the hemagglutinin and the virion itself, and, therefore, is partially lost by the usual methods of viral concentration. Optimal conditions for inducing cell fusion by Sendai virus are described.

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Effect of Regional Shielding on Plasma Enzyme Changes in Rats after 800 R X-Irradiation (33442)

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In previous studies (1–3), it was found that rats show a moderate rise in plasma glutamic oxalacetic transaminase (PGOT) at 6 hr and a marked fall in plasma alkaline phosphatase (PAkP) at 2–9 days after whole-body X-irradiation. To determine the possible origin of these plasma enzyme changes, the effect of shielding with lead various regions of the body during such an exposure was studied.

Materials and Methods.[1] Young adult male Sprague-Dawley rats weighing 200–350 g were used in a series of experiments. In each experiment, 10–14 animals were divided into a control group that was not irradiated, an irradiated group that was not shielded, and one or more regionally shielded groups. All rats in a given experiment, including nonirradiated controls, were anesthetized by intraperitoneal injection of sodium pentobarbital, 50 mg/kg, during the period of irradiation.

Irradiations were carried out at 23° with an X-ray unit operated at 300 kvp and 20

[1] The "Principles of Laboratory Animal Care" as promulgated by the National Society for Medical Research were observed during this study.