A Comparison of Immune Cytolysis and Virus-Induced Fusion of Sensitive and Resitant Cell Types* (33945)

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Previous investigations (1–3) have revealed significant biological differeces between the plasma membranes of primary rhesus monkey kidney (MK) cells and baby hamster kidney (BHK21-F) cells, particularly in their interactions with the parainfluenza virus SV5. The MK membrane is resistant to virus-induced cell fusion, whereas that of the BHK21-F cell is extremely sensitive, and detailed virological, biochemical, and morphological studies of the replication of SV5 in these two cell types have led to the conclusion that the virulence and yield of the virus depends on the response of the cell membrane to the virus (1–4). In MK cells a high yield of SV5 is produced without extensive cytopathic effect or cell death, whereas in BHK21-F cells, cell death occurs but is secondary to extensive cell fusion, and a low yield of infective virus results from a block in virus maturation at the injured cell membrane.

In addition to these differences in virus-cell interaction, other biological differences between MK and BHK21-F plasma membranes have been found. Under the same conditions of fixation, embedding, and staining, there are morphological differences between the two membranes when examined in the electron microscope (2). The MK membranes show the typical trilaminar appearance described by Robertson for "unit membrane" (5), whereas the BHK21-F cell membrane appears as a single dense line unless special staining techniques are used (2, 6, 7). The BHK21-F cell is also more susceptible to osmotic shock and to disruption by homogenization than the MK cell, and recently significant differences were found in the lipid composition of purified plasma membranes isolated from the two cell types (7).

Because of these biological and chemical differences of MK and BHK21-F cell membranes, the response of these two cell types to another membrane lesion, i.e., immune cytolysis induced by antigen–antibody–complement complexes at the cell membrane, was investigated. This communication describes studies on the lysis of uninfected cells by anti*cellular* antibodies, and of cells infected with SV5 by anti*viral* antibodies. The results indicate that there are significant differences in the inherent sensitivities of the plasma membranes of the two cell types to immune cytolysis, and that their different sensitivities to such cytolysis parallel their sensitivities to virus-induced cell fusion.

Materials and Methods. Cultivation and inoculation of cells. Primary cultures of MK cells were grown in lactalbumin hydrolysate medium with 2% calf serum, and the BHK21-F cell line was grown in reinforced Eagle's medium with 10% tryptose phosphate and 10% calf serum as described previously (1). Confluent monolayers of cells grown on glass coverslips in plastic petri dishes were inoculated with the W3 strain of SV5 (4) at multiplicities of approximately 20 pfu/BHK21-F cell and 40 pfu/MK cell, under conditions in which all cells are infected approximately simultaneously (1).

Antisera and complement. Anti-BHK21-F cell serum. Male rabbits weighing 2.5–3 kg were injected intravenously on days 1 and 8 with approximately 6 × 10^7 BHK21-F cells released from monolayers with 0.25% trypsin and 0.05% Versene. Serum was collected 17 days after the first injection.

Anti-MK cell serum. Rabbits received intravenous injections on days 1, 5, 9, 14, and 19 of 3–4 × 10^7 MK cells which had been

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frozen and thawed 5 times. Serum was collected 24 days after the first injection.

Anti-SV5 serum. Rabbits were injected with a suspension of SV5 virus grown in MK cells which contained 512 hemagglutinating units of virus/ml; 10 ml was injected intravenously on day 1, and intraperitoneally on days 2, 3, and 14. Serum was collected 27 days after the first injection. All sera were heated at 56° for 30 min before use. The *complement(C')* used was lyophilized, reconstituted guinea pig serum (Difco Laboratories, Detroit, Mich.) which had a titer of 200–400 units/ml. In experiments with cultured cells, it was used at concentrations equivalent to 10–12.5% serum, or 5–6 units/ml of medium, a total of 30–40 units of complement/monolayer of cells.

Titration of antisera. Anticellular antibodies were assayed by complement fixation tests based on the Kolmer procedure (8). Antigens were either whole MK or BHK21-F cells which had been frozen and thawed 3 times, or isolated, purified plasma membranes from MK and BHK21-F cells prepared as described in detail elsewhere (7) using a modification of the procedure of Warren and co-workers (9). Antigens prepared from the two kinds of cells were standardized to the same total protein concentration by the Lowry method (10), with bovine serum albumin as a standard. Using whole cells or plasma membranes as antigens, complement fixation tests were performed at various concentrations of antigens and antisera. With 0.3 mg/ml of cell protein as antigen, the anti-MK cell serum gave a positive complement fixation test at a dilution of 1:256, whereas the anti-BHK21-F cell serum gave a positive complement fixation test at a dilution of only 1:64. With 0.03 mg/ml of purified plasma membrane protein as antigen, the anti-MK cell serum gave a positive complement fixation test at a dilution of 1:512, and the anti-BHK21-F cell serum, 1:128. Thus, using either whole cells or isolated plasma membranes as antigen, the anti-MK cell serum had a 4-fold higher titer against an equivalent amount of cellular protein. The anti-SV5 serum had a hemagglutination-inhibition titer of 6144 against 4 hemagglutinating units of virus.

Light microscopy and photography. Cells were examined with phase contrast optics in a Zeiss Ultraphot II microscope with a 25× or 40× objective. Photomicrographs were made with Versapan or Polaroid film.

Results. Immune lysis with anticellular antibody and complement. Confluent monolayers of uninfected MK or BHK21-F cells were incubated at 37° in Eagle's minimal essential medium (MEM) (11) with various concentrations of rabbit sera and guinea pig complement. BHK21-F cells rounded up and lysed within 2 hr when incubated with anti-BHK21-F cell serum diluted 1:32 in the presence of 40 units of complement. In contrast, MK cells were *not* lysed by a 1:8 dilution of anti-MK cell serum with complement under the same conditions of incubation. In control experiments the morphology of MK or BHK21-F cells was not altered by complement or homologous antiserum alone, or by normal rabbit serum or heterologous antiserum in the presence of complement. In many experiments of this type, MK cells were consistently found to be resistant to lysis at concentrations of anticellular serum and complement which caused complete lysis of BHK21-F cells, although, as described above, the anti-MK cell serum had a higher complement fixing titer against both whole cell extracts and isolated plasma membranes than the anti-BHK21-F cell serum. These results of a typical experiment are illustrated in Figs. 1 and 2.

Although MK cells are significantly more resistant to immune cytolysis than BHK21-F cells, it should be emphasized that MK cells are lysed by anti-MK cell serum at high concentration after prolonged incubation, e.g., about 20% of the cells in a monolayer were lysed after 17-hr incubation with anti-MK cell serum diluted 1:8 and 40 units of complement.

Immune lysis with antiviral antibody and complement. SV5 is an RNA-containing virus with a lipoprotein envelope which is derived from the plasma membrane of the infected cells. Like all myxoviruses and paramyxovi-

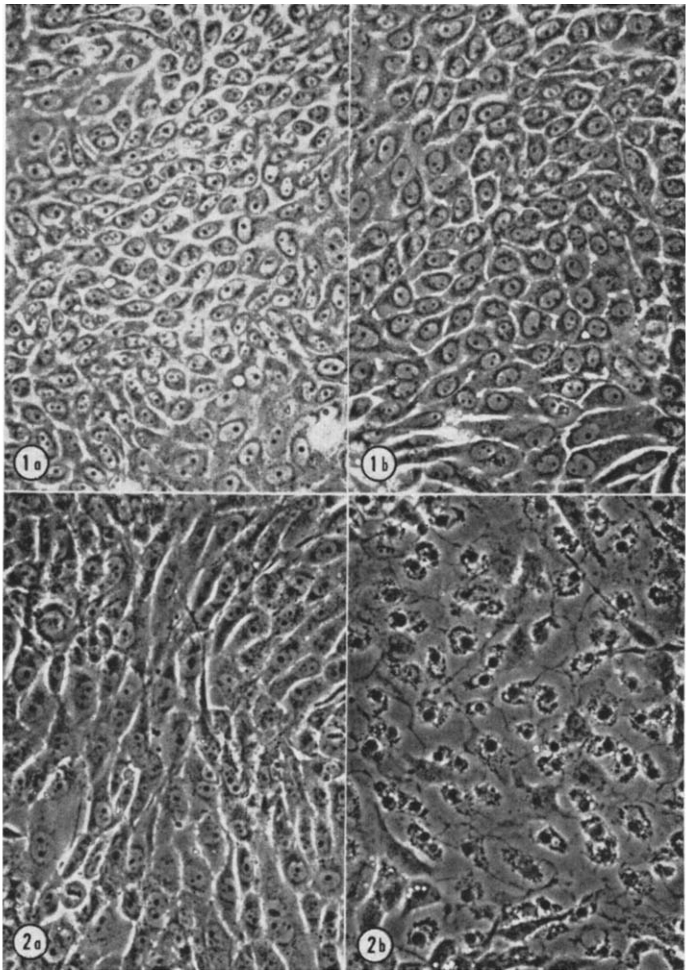


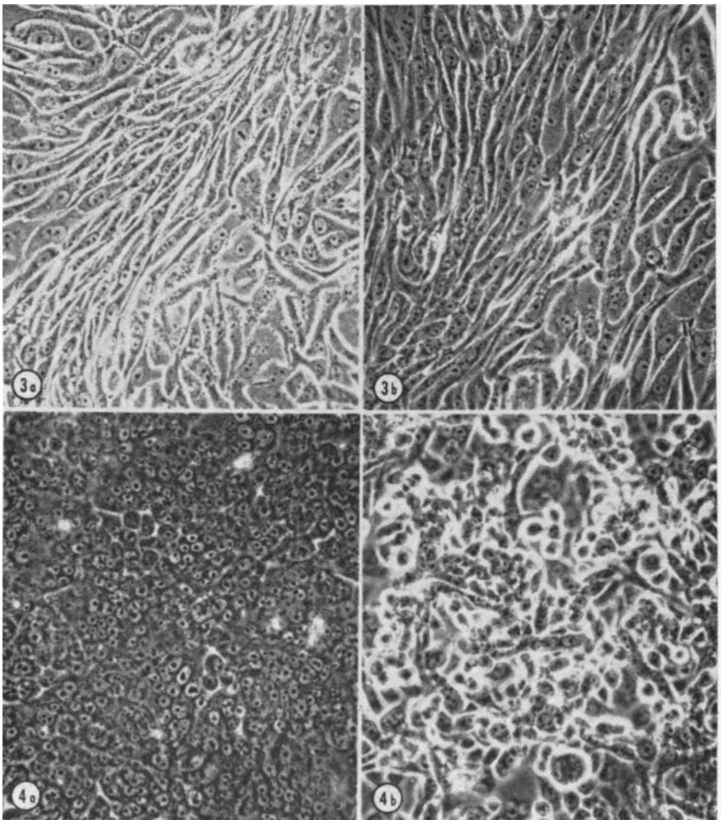
FIG. 1 and 2. Phase contrast photomicrographs; ×215.

FIG. 1. MK cells with guinea pig complement 1.75 hr after the addition of a 1:10 dilution of either normal rabbit or anti-MK cell serum: (a), normal rabbit serum added; the cells appear normal; (b), anti-MK cell serum added. The cells are morphologically indistinguishable from the control cells, indicating their resistance to immune cytolysis by anticellular antibodies and complement.

FIG. 2. BHK21-F cells with guinea pig complement 1.5 hr after the addition of a 1:20 dilution of either normal rabbit serum or anti-BHK21-F cell serum: (a), normal rabbit serum added; the cells appear normal; (b), anti-BHK21-F cell serum added; the cells have swollen and lysed.

ruses, SV5 matures by budding from regions of the membrane of the infected cell which have been altered by the addition of viral components (2). Thus, a reaction of antiviral antibody and viral antigens in such altered regions of the cell membrane in the presence of complement could lead to immune cytolysis. The responses of SV5-infected MK and BHK21-F cells to antiviral antibody and complement were therefore compared.

Monolayers of MK or BHK21-F cells were inoculated with SV5 at multiplicities of 20–50 pfu/cell. After an adsorption period of 1–2 hr at 37°, the inoculum was removed and the monolayers were washed twice with phosphate buffered saline (12). The appropriate growth medium was added, and the infected cells and uninfected controls were incubated at 37° until about 12 hr after inoculation, a time when virus maturation is



FIGS. 3 and 4. Phase contrast micrographs; ×162.

FIG. 3. SV5-infected MK cells 14 hr after infection and 2 hr after the addition of guinea pig complement and a 1:10 dilution of either normal rabbit serum or anti-SV5 serum: (A), normal rabbit serum added; the virus-infected cells appear normal; (B), anti-SV5 serum added. The virus-infected cells appear morphologically normal, indicating their resistance to immune cytolysis by antiviral antibodies and complement.

FIG. 4. SV5-infected BHK21-F cells 14 hr after infection and 2 hr after addition of guinea pig complement and a 1:10 dilution of either normal rabbit serum or anti-SV5 serum: (A), normal rabbit serum added. BHK21-F cells are very sensitive to virus-induced cell fusion and the culture consists primarily of multinucleated giant cells. Much of this SV5-induced cell fusion occurred in the 2 hr after the addition of the normal rabbit serum. (B) anti-SV5 serum added. Most of the cells are rounded up and refractile as the result of membrane damage caused by anti*viral* antibody and complement. The progression of virus-induced cell fusion was arrested by the addition of the antibody and complement.

maximal (1, 4). The cells were then treated with antiviral antiserum and complement in varying concentrations in Eagle's MEM, or the appropriate serum and complement controls, incubated at 37°, and examined by phase contrast microscopy.

In repeated experiments, all SV5-infected BHK21-F cells were rounded up or lysed within 1 hr at 37° by 1:20 diluted anti-SV5 serum plus 30–40 units of complement, but SV5-infected MK cells showed no morphological damage when treated with 1:10 diluted anti-SV5 serum plus complement. Figures 3 and 4 illustrate the results of such an experiment. Uninfected MK or BHK21-F cells were not lysed by normal serum or anti-SV5 serum with or without complement, and infected cells were not lysed by normal serum and complement, or by anti-SV5 serum without complement. It was observed that the

TABLE I. Different Sensitivities of BHK21-F and MK Cells to Immune Cytolysis.

	Complement fixation titer with homologous cell antigens		Reciprocal of serum dilution causing cell lysis[a]	
Antiserum	Whole cells[b]	Isolated plasma membranes[c]	BHK21-F cells	MK cells
Anti-BHK21-F cells	64	128	32	—
Anti-MK cells	256	512	—	<8
	Hemagglutination-inhibition titer		SV5-infected	
			BHK21-F cells	MK cells
Anti-SV5	6144		20	<10

[a] Cells incubated 2 hr at 37° with antiserum and 30–40 units of guinea pig complement.

[b] Protein concentration = 0.3 mg/ml.

[c] Protein concentration = 0.03 mg/ml.

progression of virus-induced cell fusion normally seen in SV5-infected BHK21-F cells was arrested by the addition of anti-SV5 serum with or without complement. This is probably due to the blocking by antibody of the virus-specific sites on the surface of the infected cells that are involved in cell fusion.

It should be recalled in interpreting these experiments that MK cells yield up to 1500 infective SV5 particles/cell in 24 hr (4), whereas BHK21-F cells produce only 7–10 infective particles/cell (1). Yet the BHK21-F cell is much more sensitive to immune cytolysis by antiviral antibodies and complement. Thus, whether a cell is lysed by antiviral serum depends on the inherent sensitivity of the cell membrane to immune cytolysis rather than on the amount of virus budding from the cell membrane.

The different sensitivities of the two cell types to immune cytolysis by anticellular or antiviral antibodies and the titers of the antisera against equivalent amounts of whole cell or isolated plasma membrane protein or virus are summarized in Table I. The greater sensitivity of the BHK21-F membrane to lysis by either type of antibody is apparent.

Discussion. One problem in comparing the sensitivities of different cells to immune cytolysis concerns evaluation of the potency of the respective antisera. In the present study, this was done by titering each serum against equivalent amounts of cellular protein, using not only whole cells, but also isolated, purified plasma membranes of the respective cells. The finding that with each of these antigens the MK serum gave a 4-fold *higher* titer than did the BHK21-F serum, emphasized the significance of the resistance of the MK cells and the sensitivity of the BHK21-F cell to lysis by anticellular sera. The greater sensitivity of the BHK21-F cell membrane was also clearly demonstrated by the lysis of these cells with anti-SV5 serum, under conditions in which the *same* serum did not lyse the infected MK cells . Varying sensitivity of several other cell types to immune cytolysis has been previously reported (13).

Since the relative sensitivities of BHK21-F and MK cells parallel their sensitivities to virus-induced cell fusion, the possibility should be considered that the biochemical events at the cell membranes might be similar in the two cases, but there is little evidence to support this concept. Little is known about the biochemical mechanism of virus-induced cell fusion. Fusion of cells by SV5 has a pH optimum around 8.4–8.6 as does hemolysis induced by this virus (14). Cell fusion is temperature dependent, and does not require virus replication or cellular RNA or protein synthesis (1, 14). No phospholipase activity has been associated with the virion, nor is there accelerated breakdown of cellular phospholipid during fusion (15). Further work is needed to elucidate the biochemical basis of virus-induced cell fusion.

It is of interest to consider what property of the BHK21-F cell plasma membrane makes it so sensitive to injuries such as virus-induced cell fusion and immune cytolysis, and, conversely, what property of the MK cell membrane allows it to resist these alterations. Is there a significantly different rate of membrane repair in the two cell types, or does the difference reside in the transport systems of their membranes, so that the MK cell is better equipped to maintain osmotic balance in the face of membrane injuries than is the BHK21-F cell? Another possibility is that the MK cell plasma membrane is a stronger, more rigid structure which provides protection against the injury that leads to cell fusion or the swelling, due to osmotic shock, which is the ultimate cause of immune lysis. There is some support for the latter possibility in the recent finding of significant differences between the lipid compositions of the plasma membranes of the two cell types. The MK cell plasma membranes have a significantly higher cholesterol:phospholipid ratio than BHK21-F plasma membranes (7), and it has been suggested that a high cholesterol: phospholipid ratio may account for stability of cell membranes (16, 17). There are also significant differences in the phospholipid pattern of MK and BHK21-F cell membranes.Whether those differences are responsible for the different biological behavior of the two cell types awaits further investigations.

The lysis of SV5-infected BHK21-F cells by antiviral antibodies and complement is of interest for several reasons. The immune lysis of these cells, which produce very little infective virus (1), indicates not only that the sensitivity of a given cell to lysis depends largely on the nature of the plasma membrane rather than the amount of virus budding from the membrane, but also that it is not necessary to have much virus budding from the cell membrane for cell damage to result from the reaction with virus-specific antibodies and complement. This concept is of potential importance in considering the possibilities of pathogenic immune reactions in virus infections.

It has been demonstrated previously that cells persistently infected with rabies virus are lysed by anti*viral* antibody and complement (18, 19). The present study has provided an example of this phenomenon with another type of virus. SV5 is a member of the paramyxovirus group, which includes measles, mumps, and the parainfluenza viruses, and a characteristic of viruses in this group is their ability to cause persistent, noncytocidal infections (20). An important example of what appears to be persistent infection of man by a paramyxovirus, i.e., measles, is the disease subacute sclerosing panencephalitis (21). Cells presently infected with paramyxoviruses contain viral antigens and may produce either a high yield or very little infectious virus. Immune lysis by viral antibody and complement provides a theoretically possible means of killing infected cells that might otherwise survive. It has also recently been reported that sensitized spleen cells from mumps-immune mice destroy cells persistently infected with mumps virus (22).

The present observation that MK and BHK21-F cells show different sensitivities to immune cytolysis raises the question of similar differences in sensitivity of the plasma membranes of various tissues in the body of an animal infected with a paramyxovirus, or with other viruses which mature by budding at the cell surface and which can cause persistent infection. If such differences do exist, then certain organs of the infected animal might be more susceptible to pathogenic immune reactions involving anti*viral* antiserum and complement than others.

It should also be pointed out that the ability of paramyxoviruses to damage directly cell membranes, as indicated by their cell-fusing and hemolytic activities, provides another possible mechanism for pathogenesis of diseases which involve persistent infection. This role of virus-induced injury to cell membrane in pathogenesis of virus diseases has been discussed in detail elsewhere (23, 24).

Summary. The relative sensitivities to immune cytolysis of rhesus monkey kidney (MK) cells and baby hamster kidney (BHK21-F) cells have been investigated.

BHK21-F cells are significantly more sensitive to lysis by anti*cellular* antibodies and complement than are MK cells. BHK21-F cells infected with the parainfluenza virus SV5 are susceptible to lysis by anti*viral* antibodies and complement, where MK cells are resistant. Previous studies indicated that BHK21-F cells are extremely sensitive to cell fusion induced by SV5, and that MK cells are resistant. Thus, the relative sensitivities of these two cell types to one membrane injury, immune cytolysis, parallels their sensitivities to another membrane alteration, virus-induced cell fusion. Some biological implications of these differences in the plasma membranes of the two cell types are discussed.

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