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Monoclonal Antibodies to Reovirus σ1 and μ1 Proteins Inhibit Chromium Release from Mouse L Cells

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Reovirus intermediate subviral particles (ISVPs) but not intact virions or cores have been shown to possess the capacity to permeabilize mouse L cells as determined by a ^{51}Cr release assay. We used monoclonal antibodies (MAbs) directed against proteins exposed on the ISVP surface (σ1, μ1, and λ2) to probe the role(s) of these proteins in membrane interaction and penetration. One σ1-specific MAb (MAb-G5) and two μ1-specific MAbs (MAb-10H2 and MAb-8H6) inhibited reovirus-induced ^{51}Cr release when added pre- or post-ISVP attachment to L cells. MAb-G5 inhibits ^{51}Cr release by interfering with ISVP attachment (via σ1) to L-cell receptor sites. The μ1-specific MAbs (MAb-10H2 and MAb-8H6) inhibit ^{51}Cr release by interfering with an undefined post-L-cell-attachment event that involves bivalent binding of the μ1-specific MAbs to an epitope located in a central region of the μ1 protein.

Mammalian reoviruses are nonenveloped icosahedral viruses containing a segmented double-stranded RNA genome (reviewed in references 15 and 16). Several steps in the process of reovirus entry into cells have been identified, including virion attachment to target cells, receptor-mediated internalization of virions into vesicles, acid-dependent endosomal or lysosomal protease digestion of virions to intermediate subviral particles (ISVPs), and further uncoating of ISVPs to core particles (reviewed in reference 14). We have been interested in understanding the mechanism by which reoviruses penetrate cell membranes in order to gain access to the host-cell machinery essential for virus replication. Accumulating evidence suggests that the ISVP is the form, or the immediate precursor of the form, of virus that interacts with and permeabilizes cell membranes. ISVPs generated in vitro, but not intact virions or cores, have been shown to induce the release of ^{51}Cr from preloaded L cells (1, 10), to cause hemolysis (13), and to form channels in lipid bilayers (18).

The capacity of ISVPs to interact with and permeabilize membranes suggests that one or more of the proteins exposed on the ISVP surface are directly involved in the events that result in membrane penetration during virus entry. The three proteins that are exposed on the surface of reovirus ISVPs include the σ1, μ1, and λ2 proteins. σ1 oligomers are located at the icosahedron vertices of the particle and serve as the principal viral attachment protein and hemagglutinin (9, 23). μ1 forms an outer capsid shell that covers the entire ISVP surface except where the λ2 core spikes protrude (3). The capacity of ISVPs to induce ^{51}Cr release (10) and cause hemolysis (13) has been mapped to the M2 gene (which encodes μ1), suggesting that μ1 is involved either directly or indirectly in membrane permeabilization. λ2 pentamers form spike-like structures that are associated with the core particle and protrude through the μ1 shell at the icosahedron vertices (3, 5). In this study we have used monoclonal antibodies (MAbs) specific to σ1, μ1, and λ2 to probe the role(s) of these proteins in ISVP-induced membrane permeabilization as determined by a ^{51}Cr release assay.

MAbs to reovirus ISVP surface proteins inhibit ^{51}Cr release. When type 3 strain Abney (T3A) reovirus ISVPs are added to L-cell monolayers preloaded with ^{51}Cr, up to 80% of the cell-associated ^{51}Cr is released into the overlying medium (1, 10). To determine if MAbs specific to the proteins on the surface of the ISVP can inhibit membrane permeabilization, one σ1-specific antibody (MAb-G5 [2]), four μ1-specific antibodies (MAb-10H2, MAb-8H6, MAb-4A3, and MAb-10F6 [21]), and one λ2-specific antibody (MAb-7F4 [21]) were screened for the capacity to inhibit ^{51}Cr release. ISVPs were prepared by digesting purified T3A virions with chymotrypsin as described previously (10). ISVPs were combined with protein A-purified MAbs (10 μg/ml), incubated for 1 h on ice, and then tested in a ^{51}Cr release assay identical to one previously described (method B in reference 10). The results in Fig. 1 show that MAb-G5 inhibited up to 90% specific ^{51}Cr release and two of the four μ1-specific MAbs (MAb-10H2 and MAb-8H) inhibited up to 75% specific ^{51}Cr release under the conditions of this assay. The λ2-specific MAb (MAb-7F4) did not inhibit ISVP-induced ^{51}Cr release and, indeed, appeared to slightly enhance ^{51}Cr release. A control antibody specific to type 1 reovirus σ1 (MAb-5C6 [21]), which does not bind T3A reovirus, did not inhibit ^{51}Cr release. These results indicated that the phenomenon of ^{51}Cr release by reovirus ISVPs could be inhibited by the σ1-specific MAb-G5 and by certain MAbs specific to the μ1 outer capsid protein. It should be noted that MAb-10H2 and MAb-8H6 inhibited ^{51}Cr release when the assay was performed at room temperature but not when the assay was performed at 37°C (data not shown). This difference in the capacities to inhibit ^{51}Cr release at room temperature versus 37°C could be due to the effect of temperature on the stability of the ISVP or on the fluidity of the cell membrane or both.

Effect of MAbs on particle attachment. A possible mechanism of ^{51}Cr release inhibition by MAbs is the inhibition of ISVP attachment to L cells. To test this, MAbs that inhibited ^{51}Cr release (MAb-G5, MAb-10H2, and MAb-8H6) were tested for the capacity to inhibit attachment of ^{35}S-labeled T3A ISVPs or virions to L-cell monolayers. One μ1-specific MAb that failed to inhibit ^{51}Cr release (MAb-10F6) was included as

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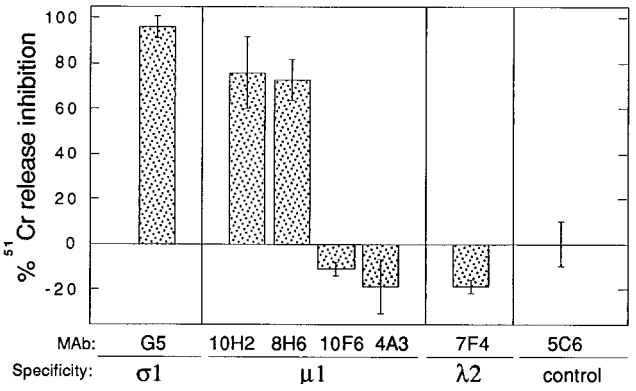

FIG. 1. ^{51}Cr release inhibition by MAbs. ISVP digests (7.5×10^{11} particles per ml) were incubated with 10 μg of the indicated MAb per ml and then tested for the capacity to induce ^{51}Cr release (150 min at room temperature; see method B in reference 10). Nonspecific release (i.e., release when 100 μl of virion storage buffer per well was added to the monolayer in place of ISVPs) was subtracted from the values, and the percentage of ^{51}Cr release inhibition by each MAb was calculated relative to release in the absence of the MAb (0% inhibition). The level of ISVP-induced ^{51}Cr release was routinely ~20,000 cpm per well, and background release was 2,000 to 3,000 cpm per well. Within each experiment MAbs were tested in duplicate. Bars represent the means of two to four separate experiments; error bars indicate standard errors of the means (SEM).

a control. ISVPs were generated from ^{35}S-labeled T3A prepared as described previously (10). ISVPs and virions were purified on sucrose gradients (10 to 40% sucrose; 75 min at 25,000 rpm in a Beckman SW28.1 rotor at 4°C) and dialyzed against virion storage buffer (150 mM NaCl, 10 mM $MgCl_2$, 10 mM Tris-HCl [pH 7.5]). Purified ISVPs or virions at 5×10^{10} particles per ml were then preincubated with the indicated concentration of MAb for 1 h on ice. This mixture (200 μl per well) was added to prechilled confluent L-cell monolayers (~2×10^6 cells per well) in 6-well plates that had previously been washed one time with ice-cold binding buffer (1% bovine serum albumin, phosphate-buffered saline [pH 7.4]). Following a 1-h incubation on ice, unattached particles were removed by being washed three times with cold binding buffer, and the monolayer was solubilized with 0.5% sodium dodecyl sulfate (SDS)–10 mM EDTA–20 mM $NaHCO_3$. The solubilized monolayer was collected, and the number of ^{35}S-labeled particles remaining associated with the monolayer were determined by scintillation counting. The results of the attachment inhibition experiments are shown in Fig. 2. MAb-G5 inhibited up to 80% virion attachment and up to 60% ISVP attachment. MAb-10H2 inhibition of virion attachment was negligible; however, this antibody inhibited ~30% ISVP attachment over a broad concentration range (0.01 to 10 μg/ml). Similarly, MAb-8H6 inhibition of virion attachment was negligible; however, this antibody inhibited ~20% ISVP attachment at 10 μg/ml. At a higher concentration (50 μg/ml), MAb-10H2 and MAb-8H6 failed to inhibit ISVP attachment. Interestingly, MAb-10F6 inhibited up to 30% virion attachment at concentrations greater than 1 μg/ml, while inhibition of ISVP attachment was negligible. MAb-4A3 failed to inhibit T3A virions or ISVPs (data not shown). The attachment inhibition results suggested that the mechanism of ^{51}Cr release inhibition by MAb-G5 involves blockage of σ1-mediated ISVP attachment to L-cell monolayers. The inhibitory effects of MAb-10H2 and MAb-8H6 on ISVP attachment were subtle; nevertheless, these MAbs did not significantly affect virion attachment, suggesting that the inhibitory effect on attachment was specific to ISVPs.

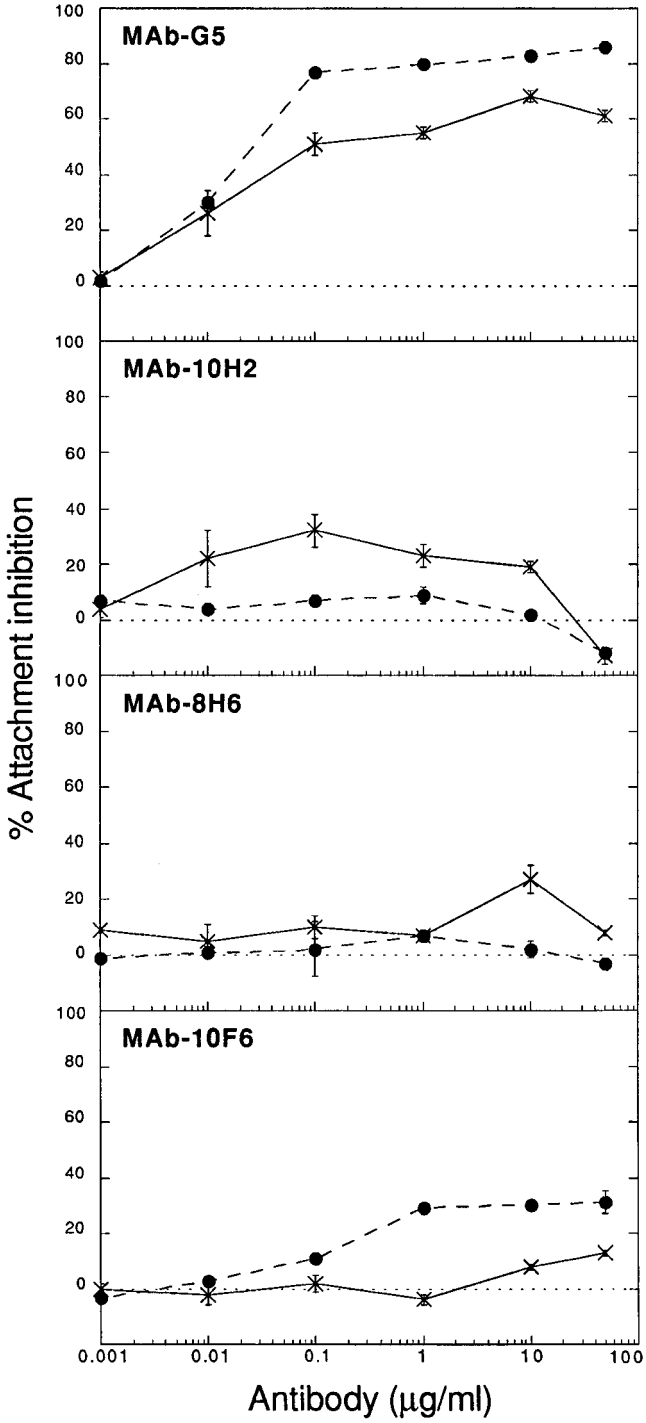

FIG. 2. Effect of MAbs on T3A ISVP or virion attachment to L-cell monolayers. The indicated concentration of each MAb was tested for the capacity to inhibit the attachment of sucrose-purified, ^{35}S-labeled T3A ISVPs (×) or virions (●) (5×10^{10} particles per ml). The percentage of attachment inhibition relative to nonspecific control antibody MAb-5C6 (0% inhibition) was calculated. Within each experiment MAbs were tested in duplicate. Each point represents the mean of two to three separate experiments; error bars indicate SEM.

^{51}Cr release inhibition by MAbs added post-ISVP attachment to monolayer. To further explore the possibility that ^{51}Cr release-inhibiting MAbs act by interfering with ISVP attachment to L cells, ^{51}Cr release inhibition experiments were per-

formed in which the antibody was added after ISVP attachment to L-cell monolayers had already occurred (post-ISVP attachment). The ^{51}Cr release assay was performed as described above; however, 30 min after the ISVPs (7.5×10^{11} particles per ml) were added to the prechilled ^{51}Cr-labeled L-cell monolayer, unattached ISVPS were removed by being washed three times with minimal essential medium (MEM)–10 mM HEPES (*N*-2-hydroxylethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 7.4). Antibody diluted in MEM–5% fetal bovine serum (cMEM) (100 μl) was added to each well and held for 30 min at 4°C to bind ISVPs. cMEM (400 μl) was added to the wells, the plates were placed at room temperature, and the ^{51}Cr release assay proceeded as previously described (10). As shown in Fig. 3, both MAb-10H2 and MAb-8H6 added post-ISVP attachment to L cells inhibited up to 80% ^{51}Cr release. MAb-10H2 exerted an inhibitory effect over a broad range of concentrations but failed to inhibit ^{51}Cr release at the highest concentration tested (100 μg/ml). MAb-8H6 at concentrations greater than 10 μg/ml inhibited up to 80% specific ^{51}Cr release. Surprisingly, MAb-G5 also inhibited ^{51}Cr release (>90%) under these conditions. The MAb-G5 results were unexpected because the attachment inhibition experiments (Fig. 2) suggested that this MAb inhibited ^{51}Cr release by blocking the attachment of ISVPs to L cells.

MAb-G5 elutes ISVPs from L-cell monolayers. One mechanism by which MAbs could inhibit L-cell-attached ISVPs from causing ^{51}Cr release would be to reverse attachment, thereby eluting ISVPs from the monolayer. To test this possibility, MAb-G5, MAb-10H2, MAb-8H6, and control MAb-5C6 were assessed for their ability to elute ^{35}S-labeled ISVPs from L-cell monolayers. ^{35}S-labeled sucrose-purified ISVPs (10^{11} particles per ml) diluted in binding buffer (4°C) (200 μl) were added to L-cell monolayers (2×10^6 cells per well of 6-well plates) that had been prechilled on ice for 10 min and washed with 1 ml of binding buffer (4°C) per well. After a 1-h incubation on ice, unattached ISVPs were removed by being washed three times with 1 ml of ice-cold binding buffer. MAbs (200 μl per well) diluted in binding buffer were added to the monolayers, and the cultures were incubated for 1 h on ice. The wells were washed as described above, and radiolabeled ISVPs remaining in the wells were collected and counted as described for the attachment inhibition assay. MAb-G5 eluted up to 30% of the attached ISVPs from the surface (Fig. 4). This result suggested that at least one way that MAb-G5 inhibits ^{51}Cr release when added post-ISVP attachment was by eluting ISVPs from the surface of the cell. The μ1-specific MAbs did not elute detectable levels of ISVPs at any concentrations tested (Fig. 4). This suggested that for μ1-specific MAbs, ^{51}Cr release is inhibited by ISVPs that remain attached to the cell monolayer.

Fab-G5, but not Fab-10H2 or Fab-8H6, added post-ISVP attachment to L cells inhibits ^{51}Cr release. In both the attachment inhibition experiment (Fig. 2) and the postattachment ^{51}Cr release experiment (Fig. 3), MAb-10H2 exhibited a notable pattern of inhibition. Inhibitory activity was evident with increasing MAb concentrations but then decreased at higher concentrations. MAb-8H6 yielded similar results in the ISVP attachment inhibition experiment (Fig. 2). One possible explanation for these results is that these MAbs exert an inhibitory effect when bound bivalently to epitopes located on either the same particle (intraparticle cross-linking) or separate particles (interparticle cross-linking [aggregation]). At high antibody concentrations, bivalent binding would be supplanted by monovalent binding, thus the prozone effect.

To test the possibility that bivalent binding is required for ^{51}Cr release inhibition, Fab fragments of ^{51}Cr release-inhibiting MAbs (MAb-G5, MAb-10H2, and MAb-8H6) were made by digestion with immobilized papain as described by the manufacturer (ImmunoPure Fab Kit; Pierce, Rockford, Ill.), subjected to SDS-polyacrylamide gel electrophoresis (PAGE) to confirm successful digestion (data not shown), and tested for

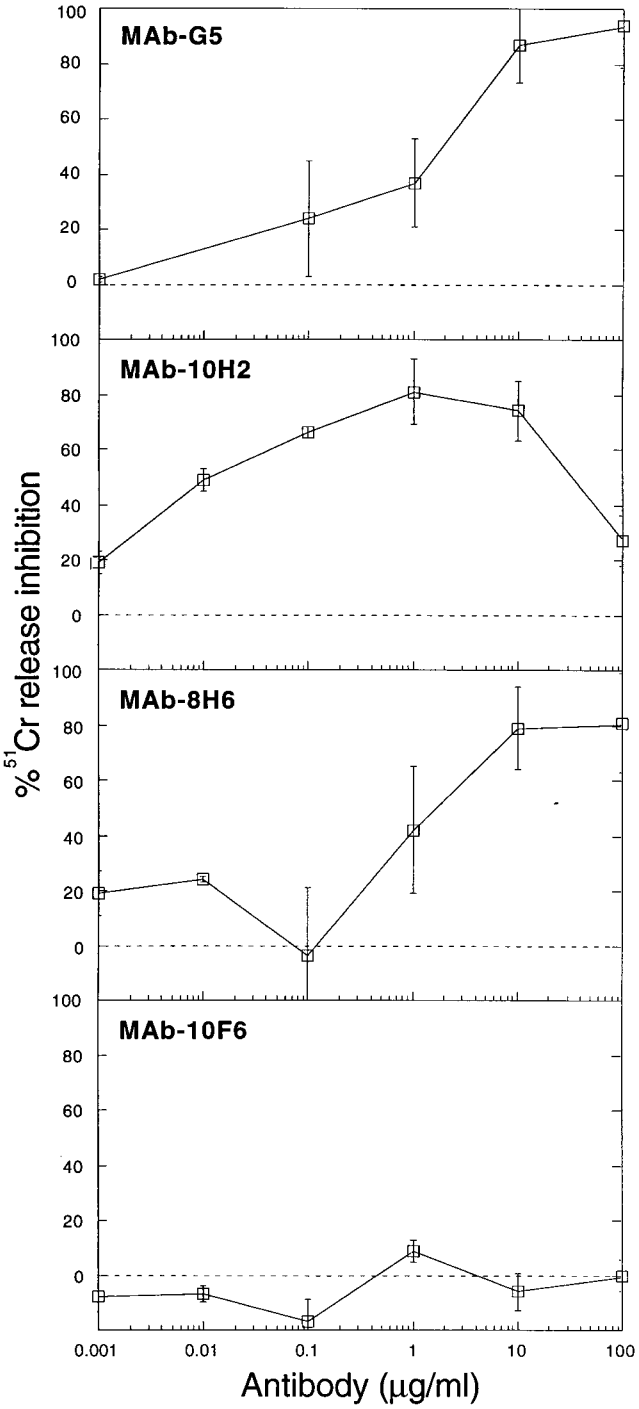

FIG. 3. ^{51}Cr release inhibition by MAbs added post-ISVP attachment to L-cell monolayers. The indicated concentration of each MAb was tested for the capacity to inhibit ISVP-induced ^{51}Cr release when added after ISVP attachment (at 4°C) to the L-cell monolayer. The amount of ^{51}Cr release was determined after 120 min at room temperature. Nonspecific release (release in the absence of ISVPs) was subtracted from the values, and the percentage of ^{51}Cr release inhibition by each MAb was calculated relative to release in the absence of MAb (0% inhibition). Within each experiment MAbs were tested in duplicate. Bars represent the means of two separate experiments; error bars indicate SEMs.

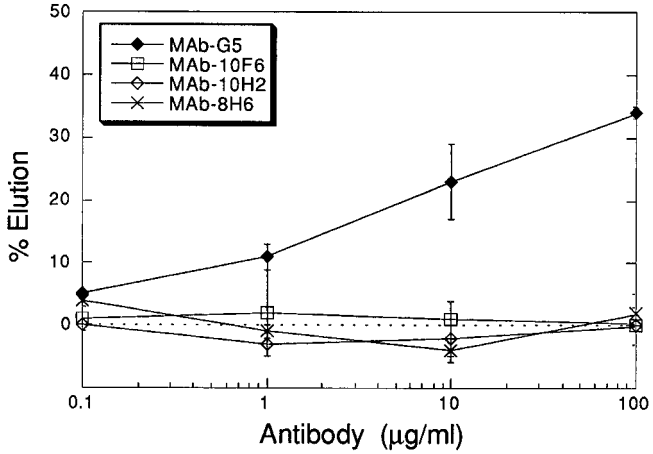

FIG. 4. Capacity of MAbs to elute T3A ISVPs from L-cell monolayers. The indicated concentration of each MAb was tested for the capacity to elute ISVPs from L-cell monolayers as described in the text. The percentage of ISVPs eluted was determined relative to elution in the presence of control antibody MAb-5C6 (elution by MAb-5C6 was set to 0%). Within each experiment samples were tested in duplicate. Each point represents the mean of two separate experiments; error bars indicate SEMs.

inhibitory activity in a postattachment ^{51}Cr release inhibition assay. As predicted, neither Fab-10H2 nor Fab-8H6 significantly inhibited ISVP-induced ^{51}Cr release at the concentrations tested (Fig. 5). In contrast, Fab-G5 inhibited ^{51}Cr release to approximately the same extent as that observed for intact MAb-G5. This finding suggested that MAb-G5 inhibits ^{51}Cr release by masking a region of σ1 directly involved in interaction with L cells. On the other hand, MAb-10H2 and MAb-8H6 inhibition of ^{51}Cr release requires either the Fc portion of the molecule (e.g., for steric hindrance) or the capacity to bind μ1 epitopes bivalently or both.

F(ab′)$_2$-10H2 and F(ab′)$_2$-8H6 added post-ISVP attachment to L cells inhibit ^{51}Cr release. To test the possibility that MAb-10H2 or MAb-8H6 inhibition of ^{51}Cr release requires either the Fc portion of the antibody or the capacity to bind bivalently, F(ab′)$_2$ was made by digesting MAbs with immobilized pepsin as described by the manufacturer (ImmunoPure F(ab′)$_2$ kit; Pierce). F(ab′)$_2$ was purified by using protein A-agarose-affinity columns (Pierce), and the purity of the fragments (absence of intact immunoglobulin) was determined by SDS-PAGE (data not shown). F(ab′)$_2$ was tested for inhibitory activity in a postattachment ^{51}Cr release inhibition assay. Both μ1-specific F(ab′)$_2$s inhibited ^{51}Cr release when added post-ISVP attachment (Fig. 5). The pattern of inhibition was identical to the pattern of the intact MAb; however, the percent inhibition was less, possibly because of contaminating Fab fragments which would compete for binding sites and prevent MAb bivalent binding. In support of this, Fab-10H2 added to ISVPs prior to adsorption diminished postattachment ^{51}Cr release inhibition by MAb-10H2 and MAb-8H6 (data not shown). These results implied that the Fc portion of MAb-10H2 and MAb-8H6 is not required for ^{51}Cr release inhibition (i.e., steric hindrance by Fc region of the antibody). Thus, the mechanism of MAb-10H2 and MAb-8H6 inhibition of ISVP-induced ^{51}Cr release involves bivalent binding of the MAbs to μ1 epitopes exposed on the surface of ISVPs.

Mechanism of ^{51}Cr release inhibition by MAb-G5. Our results demonstrate that σ1-specific MAb-G5 inhibits ISVP-induced ^{51}Cr release by interfering with ISVP attachment to L cells. Previous studies have shown that MAb-G5 blocks attachment of type 3 strain Dearing virions to L cells, and attachment inhibition has been proposed to be the mechanism by which this antibody neutralizes virus in tissue culture and protects mice in vivo (22). Others have demonstrated that Fab fragments of MAB-G5 block type 3 Dearing virion attachment to endothelial cells (20). We have found that extended proteolytic digestion of type 3 Dearing ISVPs results in the removal of σ1 and this loss correlates with a loss in capacity of the particle to cause ^{51}Cr release (6). Together these data strongly suggest that ISVP attachment to L cells via σ1 is a prerequisite for ISVP-induced ^{51}Cr release under the conditions of this assay. Attachment is not a prerequisite for other membrane permeabilization assays involving reovirus ISVPs including channel formation in lipid bilayers (18) and hemolysis (13). Thus, the ^{51}Cr release assay used in these experiments involves specific

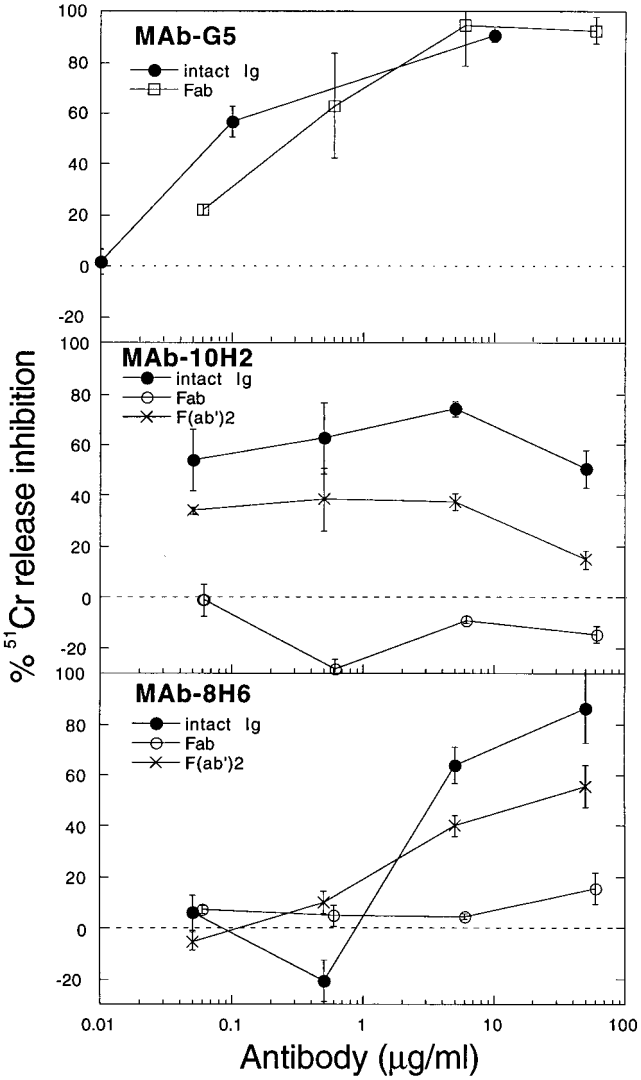

FIG. 5. ^{51}Cr release inhibition by Fab and F(ab′)$_2$ added post-ISVP attachment to L-cell monolayers. The indicated concentrations of MAbs, Fabs, or F(ab′)$_2$s were tested for the capacity to inhibit T3A ISVP-induced ^{51}Cr release when added post-ISVP attachment to the monolayer. Nonspecific release (release in the absence of ISVPs) was subtracted from the values, and the percentage of ^{51}Cr release inhibition by each MAb, Fab, or F(ab′)$_2$ was calculated relative to release in the absence of antibody (0% inhibition). Within each experiment samples were tested in duplicate wells. Each point represents the mean of two separate experiments; error bars indicate SEMs.

ISVP interactions with the cell surface (i.e., attachment via the σ1 protein) followed by interactions that result in permeabilization of the cell membrane.

When MAb-G5 was added post-ISVP adsorption to L cells, ISVP-induced ^{51}Cr release was inhibited. This result suggested that MAb-G5 could either reverse ISVP attachment or interfere with a subsequent event required for ^{51}Cr release. Elution experiments indicated that approximately one-third of the attached ISVPs were released into the overlying medium when 100 μg of MAb-G5 per ml was added (Fig. 4), suggesting that MAb-G5 can reverse ISVP attachment. However, because the number of ISVPs eluted account for only ~30% of the observed loss in the capacity to induce ^{51}Cr release, it is likely that MAb-G5 can also act at a step subsequent to the initial ISVP attachment to L cells. It is possible that multiple σ1 oligomers may be required to attach surface receptors before ISVP-induced ^{51}Cr release can occur. If this were the case, MAb-G5 added post-ISVP attachment to L cells could inhibit ^{51}Cr release both by eluting ISVPs and by preventing the remaining bound ISVPs from making multivalent σ1 contacts with L-cell receptors as the monolayer warmed. Alternatively, it is possible that σ1-bound MAb-G5 sterically hinders a non-σ1-mediated ISVP–L-cell interaction; however, since Fab fragments of MAb-G5 inhibited ^{51}Cr release, steric hindrance is a less likely possibility.

Mechanism of ^{51}Cr release inhibition by MAb-10H2 and MAb-8H6. Two of the four μ1-specific MAbs that were tested inhibited ^{51}Cr release when they were added pre- or post-ISVP attachment to L cells. These MAbs (MAb-8H6 and MAb-10H2) share several properties including the same immunoglobulin G2a isotype (21), coinciding epitopes (cross-competing in an enzyme-linked immunosorbent assay [ELISA] [21]), and a high degree of binding reactivity to a T3A ISVP antigen as determined by ELISA (data not shown). However, it should be noted that these MAbs differ from each other in several properties, including the capacity to alter plaque formation (in standard plaque neutralization assays, MAb-8H6 and MAb-10F6 reduced the number of T3A plaques by up to 40% and reduced plaque size, whereas MAb-10H2 and MAb-4A3 had no effect on the number of plaques or plaque size [data not shown]) and the capacity to protect mice from reovirus challenge (MAb-8H6 protects mice from intracranial, intramuscular, and peroral challenge, whereas MAb-10H2, MAb-10F6, and MAb-4A3 are not protective [19]).

At optimal concentrations, both MAb-8H6 and MAb-10H2 appeared to inhibit some ISVP attachment to L cells. However, this effect was not sufficient to account for the ^{51}Cr release inhibition observed in Fig. 1. Moreover, these μ1-specific MAbs inhibited ^{51}Cr release when they were added after ISVPs had attached to L cells, and unlike σ1-specific MAb-G5, MAb-10H2 and MAb-8H6 failed to elute ISVPs from L-cell monolayers. Together these data indicate that, in addition to a minor effect on ISVP attachment, the μ1-specific MAbs inhibit ^{51}Cr release by interfering with an undefined postattachment event(s).

The prozone effect observed for MAb-10H2 in the ^{51}Cr release inhibition assay suggested that the inhibitory effect of this antibody requires bivalent binding. The failure of Fab fragments of MAb-10H2 and MAb-8H6 to inhibit ISVP-induced ^{51}Cr release supports the hypothesis that bivalent MAb binding is required for inhibition. Interparticle cross-linking has not been ruled out as the mechanism by which the μ1-specific MAbs inhibit ^{51}Cr release. However, ISVPs coated with MAb-10H2 or MAb-8H6 (under conditions with which ISVP-induced ^{51}Cr release is inhibited) were not found as aggregates when visualized by electron microscopy (data not shown). Furthermore, the fact that MAb-10H2 and MAb-8H6 inhibited ^{51}Cr release when they were added post-ISVP attachment to L cells implies that if these MAbs inhibit ^{51}Cr release by aggregating ISVPs, then they do so by cross-linking particles attached to the cell surface. Although it is possible that interparticle cross-linking on the cell surface inhibits ISVP-induced ^{51}Cr release, we favor the alternative possibility that MAb-10H2 and MAb-8H6 inhibit ^{51}Cr release by cross-linking epitopes on the same particle (intraparticle cross-linking). Intraparticle cross-linking could mask a region of the ISVP required for interaction with the cell, stabilize the particle preventing essential conformational change(s), or alter the conformation of the particle rendering it incapable of membrane interaction and penetration. Intraparticle cross-linking of epitopes on the surface of other nonenveloped viruses, including picornaviruses (8, 11, 17), has been shown to be one mechanism by which an antibody neutralizes infectivity. Uranyl formate-stained electron micrographs of T3A ISVPs incubated with MAb-10H2 under conditions identical to those in which ^{51}Cr release is inhibited suggest that MAb-10H2 binds symmetric locations on the particle surface, possibly around the icosahedral vertices (data not shown). It will be of interest to obtain cryoelectron microscopy image reconstructions of the MAb-10H2–ISVP complexes. Such images would confirm or refute the occurrence of intraparticle bivalent binding and would also define the topographical location of the epitope recognized by this MAb.

We have recently obtained genetic evidence that sequences in a central region of μ1 play a role in dictating the capacity of ISVPs to cause ^{51}Cr release and have proposed that this region may regulate the conversion of ISVPs to core particles (6). Interestingly, the epitope(s) recognized by MAb-10H2 and MAb-8H6 is contained within the aforementioned central region of μ1, between amino acids 285 and 506 (7). MAb-4A3 also binds this region of μ1; however, this antibody binds T3A virions and ISVPs with a low degree of efficiency, as determined by ELISA (data not shown), which may explain why it fails to inhibit ^{51}Cr release. MAb-10F6 binds T3A virions and ISVPs with a high degree of efficiency, as determined by ELISA (data not shown), and yet fails to inhibit ^{51}Cr release, possibly because it binds a different region of μ1, located between amino acids 462 and 581 (7).

It is possible that the membrane permeabilization detected in the ^{51}Cr release assay is the result of an ISVP conformational change. MAb-10H2 and/or MAb-8H6 could inhibit ^{51}Cr release by interfering with ISVP conformational changes by either stabilizing the particle (e.g., cross-linking epitopes) or by blocking ISVP-cell interactions that trigger conformational change. Alternatively, the μ1-specific MAbs could inhibit ^{51}Cr release by directly interfering with regions of the ISVP that insert into the cell membrane during penetration.

Although the ^{51}Cr release assay used in this study does not necessarily determine penetration and entry of viral particles into cells, it does determine specific ISVP-cell interactions that result in permeabilization of the membrane. This assay has been used to identify the ISVP form of reovirus as the form capable of permeabilizing cell membranes (1, 10); it has been used to identify the M2 gene product, μ1, as an important determinant in the capacity of ISVPs to permeabilize membranes (10); and it has been used to demonstrate the importance of the central region of μ1 in the capacity of ISVPs to permeabilize membranes (6). The ^{51}Cr release assay together with other assays that determine nonenveloped virus interaction with membranes, including hemolysis assays (13), liposome lysis assays (12), channel formation in lipid bilayers (18), and a recently described virus-induced fusion-from-without as-

say (4), should help us elucidate the mechanism(s) by which nonenveloped viruses penetrate cell membranes.

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