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Uptake of Reovirus Serotype 1 by the Lungs from the Bloodstream Is Mediated by the Viral Hemagglutinin

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We used the mammalian reoviruses to determine the molecular basis of the clearance of a virus from the bloodstream by specific organs. Reovirus serotypes 1 (T1) and 3 (T3) were radiolabeled with [^{35}S]methionine or ^{125}I, and the viruses were injected intravenously into weanling rats. The distribution of radioactivity within the animals was determined at various times after the injection. Both viruses were cleared rapidly from the bloodstream and concentrated in different organs. Reovirus T1 was found predominantly in the lungs and liver, whereas T3 was found predominantly in the liver, with very little virus in the lungs. Using intertypic reassortants, we determined that the T1 S1 gene, which encodes the viral hemagglutinin (σ1 protein), is responsible for the difference in uptake of T1 and T3 by the lungs. The genetic mapping was extended by using several approaches. (i) T1 subjected to limited proteolytic digestion with chymotrypsin was cleared efficiently by the lungs despite the removal of σ3 and digestion of μ1C to δ. (ii) Uptake of T1 by the lungs was totally inhibited by incubation of T1 with an anti-σ1 monoclonal antibody or its Fab fragment before injection. (iii) A reovirus T1 variant in the σ1 protein was poorly taken up by the lungs. These data indicate that clearance of reovirus from the bloodstream by the lungs is dependent on the presence of the T1 σ1 protein.

Viruses are capable of entering the bloodstream of a host by direct introduction through the skin or mucous membranes or after an initial stage of replication at the site of entry into the organism. Once in the bloodstream, viruses come into contact with a variety of cells, including those lining the vascular bed of various organs and those within the circulation. The result of interaction between viral particles and these cells is important in determining the ability of the particles to establish a productive infection within a host, especially if organs distant from the site of viral entry into the organism are to become infected. The mechanisms of viral clearance, defined as removal of circulating viruses from the bloodstream by peripheral organs, are poorly understood. Most viruses are rapidly removed from the circulation by the liver and spleen, and clearance of viruses, as with most particulate substances, is primarily dependent on the size of the viral particles, since larger particles are removed faster from the bloodstream than are smaller particles (15).

To understand which viral components are involved in the clearance of viruses from the circulation, we studied the fate of the mammalian reoviruses after intravenous inoculation. Reoviruses are icosahedral (70-nm diameter), nonenveloped viruses with a genome composed of 10 segments of double-stranded RNA (11, 28). They are divided by neutralization and hemagglutination tests into three distinct serotypes: 1, 2, and 3 (19). Despite similar outer capsid structures and genome organizations, the three serotypes exhibit unique biologic properties. The ability to generate reassortant viruses among the three serotypes has allowed mapping of distinct biologic properties to individual gene segments (11, 22, 28).

We have reported that radiolabeled reovirus serotypes 1 (T1) and 3 (T3) are rapidly cleared from the circulation after intravenous injection in adult rats (30); T1 was taken up by the lungs and liver, whereas T3 was taken up predominantly by the liver, with very little virus sequestered by the lungs (30). In this study, we used a variety of approaches, including genetic analysis, to demonstrate that clearance of T1 by the lungs is dependent on a single viral protein, the viral hemagglutinin. This study demonstrates that clearance of a virus from the bloodstream can be dependent on the specific interaction between a discrete viral protein and specific target tissues.

MATERIALS AND METHODS

Virus and cell culture conditions. Reovirus T1 strain Lang and T3 strain Dearing were used in these studies. Intertypic reassortant viruses derived from these two viruses were prepared by E. Brown, M. Nibert, and D. Drayna (5; unpublished data). Viruses 1HA3 and 3HA1 were isolated and characterized by Weiner et al. (32). The T1 mutant B9B was selected by its ability to grow and form plaques in the presence of neutralizing concentrations of a T1-specific monoclonal anti-σ1 antibody (S. Lynn and B. N. Fields, unpublished data). Virus titers were determined by plaque assay on mouse L cells (27). The hybridomas 5C6 (Lynn and Fields, unpublished data) and G5 (1) were grown in RPMI 1640 supplemented with 10% fetal bovine serum in a 95% air–5% CO_2 atmosphere at 37°C.

Animals. Rats used in these studies were 22- to 30-day-old male Sprague-Dawley rats (Charles River Laboratory, Wilmington, Mass.) whose sera did not contain detectable reovirus-neutralizing activity when assayed as previously described (1). All rats were used within 3 days of arrival and fasted overnight before an experiment.

Virus-labeling procedures. Viruses were biosynthetically labeled by addition of 20 to 40 μCi of [^{35}S]methionine (Amersham Corp., Arlington Heights, Ill.) per ml to mouse L cells grown in suspension (5) 8 hours after the initiation of infection (multiplicity of infection, 10). The cells were harvested 48 to 72 h after inoculation, and the virus was purified

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by CsCl density gradient centrifugation after Freon extraction as previously described (23). The specific activities of these viral preparations varied from 2.5 to 9.0 nCi/μg of protein, with an average particle-to-PFU ratio of 38.

Viruses were iodinated with Bolton-Hunter reagent. Cesium chloride-purified reovirus particles (50 to 100 μg of protein) were incubated with 1 mCi of ^{125}I-labeled Bolton-Hunter reagent (4,000 Ci/mmol; Amersham) in 50 μl of 0.05 M sodium Borate buffer (pH 8.3) for 1 h at 4°C. The reaction was terminated by addition of 500 μl of 0.2 M glycine in phosphate-buffered saline (pH 7.4). Unreacted Bolton-Hunter reagent was separated from labeled virus by chromatography on a Sephadex G-50 column equilibrated with phosphate-buffered saline containing 0.25% (wt/vol) gelatin (Fisher Scientific Co., Medford, Mass.). The specific activity of the virus varied from 380 to 1,160 nCi/μg of protein. This corresponds to addition of 13 to 40 molecules of Bolton-Hunter reagent per viral particle. Iodination of the virus was associated with a three- to eightfold decrease in infectivity (particle/PFU ratio, 305) as assessed by plaque assay on L-cell monolayers. By using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (17), followed by autoradiography and densitometry scanning, we found that the pattern of incorporation of label was the same as we have previously reported (14). Whereas 95% of the ^{125}I was associated with two proteins of the outer capsid of the virion, σ3 (74%) and μ1c (21%), σ1 did not incorporate enough label to be detected. The λ2 protein, a protein projecting from the core to the surface of the virus, contained approximately 2% of the label. More than 95% of the radioactivity associated with the ^{125}I- and ^{35}S-labeled viruses was precipitable with cold 10% (wt/vol) trichloroacetic acid.

Preparation of ISVPs. ^{35}S-labeled T1 intermediate subviral particles (ISVPs) were prepared by incubation of ^{35}S-labeled T1 (10^{12} viral particles) with chymotrypsin (100 μg/ml; Sigma Chemical Co., St. Louis, Mo.) in 1 ml of 150 mM NaCl–15 mM $MgCl_2$–10 mM Tris hydrochloride (pH 7.4) for 30 min at 37°C (26). The reaction was terminated by addition of phenylmethylsulfonyl fluoride (Sigma), and the sample was applied to a 1.2- to 1.5-g/ml CsCl density gradient and centrifuged at 100,000 × *g* for 3 h. A control reaction without chymotrypsin digestion was also performed. From the sample treated with chymotrypsin, particles with a density of 1.4 g/ml were collected and dialyzed at 4°C against three changes of dialysis buffer (150 mM NaCl, 15 mM $MgCl_2$, 10 mM Tris hydrochloride [pH 7.4]). From the sample not treated with chymotrypsin, particles with a density of 1.34 g/ml were similarly prepared. Chymotrypsin digestion of reovirus T1 resulted in a threefold increase in infectivity.

Purification of monoclonal antibody and monovalent Fab fragments. Monoclonal antibodies 5C6 and G5 were purified from culture supernatant fluids by passing of the fluid through a protein A-Sepharose affinity column (Pharmacia, Uppsala, Sweden) and elution with 50 mM glycine (pH 2.3). The eluted protein was neutralized and dialyzed overnight against phosphate-buffered saline at 4°C. Fab fragments of the antibody were generated by the following method (25). Affinity-purified antibody (5 mg) in 1 ml of 0.1 M phosphate buffer (pH 7.5) was incubated for 4 h at 37°C in the presence of 2% (wt/wt) mercuripapain (Worthington Diagnostics, Freehold, N.J.) and 10 mM β-mercaptoethanol. The reaction was quenched with iodoacetamide (50 μl of a 0.3 M solution in 1 M Tris hydrochloride [pH 8]). The preparation was maintained on ice for 30 min followed by dialysis against three changes of buffer (5 mM Tris hydrochloride [pH 8]). Fab fragments were purified by passing of the digestion mixture over a protein A-Sepharose affinity column and collection of the material not retained by the affinity matrix. The digestion reaction was essentially complete as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (data not shown).

Injection protocol. Radiolabeled virus, diluted in phosphate-buffered saline containing 0.1% bovine serum albumin to a final specific activity of 1,000 cpm/μl (2,500 cpm/g of body weight), was injected into the tail veins of rats under light ether anesthesia. After various periods, the animals were killed by decapitation, blood was collected in heparinized tubes, and various organs were dissected and stored at −20°C until processed. In experiments involving 5C6 or 5C6 Fab, the radiolabeled virus and the antibody (final concentration, 10 μg/ml) were incubated overnight at 4°C and injected together as described above.

Processing of tissue samples for radioactivity measurement. When ^{125}I-labeled viruses were used, organs were thawed and a small piece of tissue was weighed and counted with a gamma counter. A sample of blood (200 to 500 μl) was also counted. In all experiments, at least four rats per time point were used. When ^{35}S-labeled viruses were used, 100 to 200 mg of each organ was digested in 1.5 ml of Protosol (New England Nuclear Corp., Boston, Mass.) for 12 h in a shaking water bath at 55°C. To eliminate color quenching, each sample was bleached by addition of 450 μl of a 0.83 M solution of benzoyl peroxide in toluene (Aldrich Chemical Co., Inc., Milwaukee, Wis.), and the samples were incubated for 1 h in a shaking water bath at 55°C. Aquasol II (15 ml; New England Nuclear) was added, and the sample was counted 2 days later, after the chemiluminescence was allowed to decay to less than 1% of the total counts. Results were corrected for quenching by use of a standard quenching curve and the external standard ratio method (Rackbeta II; LKB, Turku, Finland). Blood samples (200 μl) were incubated for 1 h at 60°C in 500 μl of a 1:3 dilution of Protosol in ethanol. Samples were decolorized by dropwise addition of 500 μl of a 30% solution of hydrogen peroxide (Fisher Scientific) and incubation at 60°C for 30 min in a shaking water bath. Samples were counted with a scintillation counter after neutralization with 500 μl of 0.5 N HCl and addition of 15 ml of Aquasol II.

RESULTS

Time course and organ specificity of virus clearance. To determine the time course of clearance of ^{35}S-labeled T1 and ^{35}S-labeled T3, both viruses were injected intravenously and the animals were killed after 5, 20, or 60 min. Both T1 and T3 were cleared rapidly from the bloodstream. At 5 min after injection, specific activity in blood was 3.2 cpm/mg for both viruses, whereas the radioactivity content of several organs had already increased to a maximum (Fig. 1). At 20 min after the injection, T1 was found predominantly in the lungs, liver, and spleen, whereas other organs, such as the brain and kidneys, contained small amounts of radioactivity (specific activity, <5 cpm/mg; Fig. 1). Reovirus T3 was taken up predominantly by the liver and spleen, and very little virus was found in the lungs or other organs analyzed. The liver was the major site of clearance for both T1 (49% of the injected dose) and T3 (83% of the injected dose) (Table 1). The lungs were the second most important site of clearance for T1 (37.2% of the injected dose) but accounted for only 1.1% of the T3 virus injected. The spleen, characterized by a high specific activity for both T1 and T3, cleared only 1.9% of the total dose of T1 and 3.7% of the total T3 dose. Less

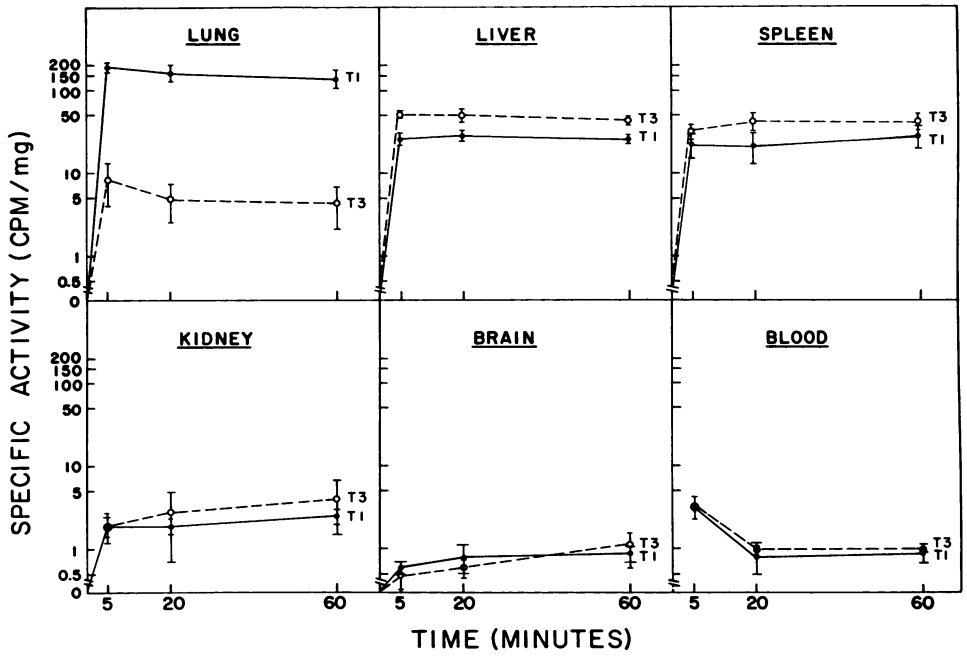

FIG. 1. Time course of clearance of ^{35}S-labeled reovirus T1 and T3. Radiolabeled virus was injected into the tail veins of anesthetized rats. At various time points, the animals were sacrificed and the radioactivity associated with specific organs was determined. The results are presented as specific activities as a function of time (mean ± 1 standard deviation; ^{35}S-labeled T1, n = 6; ^{35}S-labeled T3, n = 7).

than 2% of the total dose injected in the bloodstream was present in that compartment 20 min later. The contribution of the lungs, liver, spleen, kidney, and blood accounted for 90% of the injected T1 virus and 91% of the injected T3 virus (Table 1).

Since the amount of ^{35}S-labeled virus injected in these experiments was quite large (~6 × 10^{10} virus particles) and might saturate specific clearance sites, the clearance of ^{125}I-labeled reovirus, with a specific activity 100 times higher than that of ^{35}S-labeled virus, was analyzed. A comparison of specific activities of various organs after injection of the same amount of radioactivity of either ^{35}S- or ^{125}I-labeled viruses is presented in Table 1. The results obtained with both labeling techniques are similar, indicating that the data obtained with ^{35}S-labeled virus were representative of situations in which a low concentration of virus gains access to the bloodstream. For this reason and since ^{35}S-labeled viral preparations were more stable, ^{35}S-labeled viruses were used in the studies described below.

Genetic mapping of the gene responsible for T1 uptake by the lungs. Intertypic reassortant viruses were used to determine the genetic basis for the difference in uptake between T1 and T3 by the lungs. In view of the rapidity of virus uptake by the lungs (less than 10 min; 30), it was assumed that the difference in uptake of T1 versus T3 by the lungs was mediated by one or several of the outer capsid proteins of the virion: λ2, μ1C, σ1, and σ3. Parental and reassortant viruses representing 13 of 16 possible permutations of the outer capsid proteins between T1 and T3 were selected and used in this study (Table 2). All viruses were biolabeled with [^{35}S]methionine, purified, and injected as described in Materials and Methods. The rats were killed 20 min after intravenous injection, and the organs were processed for radioactivity measurement. When compared on the basis of

TABLE 1. Comparison of the clearances of ^{35}S-labeled and ^{125}I-labeled reovirus T1 and T3 by various organs 20 min after injection

Organ	% of total body weight[a]	% of dose injected[b]		Mean (±1 SD) sp act (cpm/mg of tissue)[c]			
		T1	T3	T1		T3	
				^{35}S	^{125}I	^{35}S	^{125}I
Lung	0.56	37.2	1.1	166 ± 34	109 ± 37	5 ± 7	3 ± 1
Liver	4.15	49.0	83.0	29 ± 4	25 ± 10	50 ± 9	51 ± 5
Spleen	0.21	1.9	3.7	22 ± 9	12 ± 4	44 ± 10	11 ± 8
Kidney	0.76	<1	<1	2 ± 0	2 ± 0	3 ± 2	3 ± 1
Brain	1.79	<1	<1	<1	<1	<1	<1
Blood	4.95	1.6	2.0	1 ± 0	1 ± 0	1 ± 0	<1

[a] The parentages of total body weight were determined experimentally by weighing the organs from 10 animals and calculating the percentage of the total animal weight. The value used for the blood is described by Caster et al. (3). The total percentage of body weight was 10.63%.

[b] Specific activities were obtained by using six ^{35}S-labeled-virus-injected animals and four ^{125}I-labeled-virus-injected animals.

[c] Percentages of the dose injected were calculated by multiplying the specific activity by the weight of the organ and dividing by the total counts per minute injected into the animal. The data presented are for the clearance of ^{35}S-labeled virus only. The total percentages of T1 and T3 clearance were 90.3 and 90.7%, respectively.

TABLE 2. Lung clearance of ^{35}S-labeled reoviruses T1 and T3 and intertypic reassortants

Virus[a]	Parental origin of genome segment[b]										Mean (±1 SD) lung sp act (cpm/mg of tissue)[c]
	Outer capsid[d]				Core[d]				Nonstructural		
	S1	S4	M2	L2	S2	M1	L1	L3	S3	M3	
HLU											
T1 (Lang)	1	1	1	1	1	1	1	1	1	1	158 ± 38
3HA1	1	3	3	3	3	3	3	3	3	3	60 ± 4
H24	1	3	1	1	1	1	1	1	1	1	128 ± 20
EB1	1	1	3	3	1	1	1	1	1	1	164 ± 36
EB93	1	1	1	1	1	1	1	1	3	1	159 ± 20
EB98	1	3	1	3	3	1	1	1	1	1	127 ± 9
EB126	1	1	1	3	3	3	3	3	3	1	127 ± 38
EB144	1	1	3	1	1	1	1	1	3	3	177 ± 16
LLU											
T3 (Dearing)	3	3	3	3	3	3	3	3	3	3	5 ± 2
1HA3	3	1	1	1	1	1	1	1	1	1	5 ± 1
EB73	3	1	3	3	3	3	3	3	1	1	5 ± 2
EB138	3	1	3	1	3	1	3	1	1	1	4 ± 1
EB143	3	1	1	1	1	1	3	1	1	1	5 ± 2
E3	3	3	1	3	3	3	3	3	3	3	3 ± 1
G2	3	1	1	3	1	1	1	1	1	1	6 ± 2

[a] HLU, High lung uptake; LLU, low lung uptake.
[b] A 1 indicates that the genome segment was derived from T1, and a 3 indicates that the genome segment was derived from T3.
[c] Specific activities in the lungs were determined by using 5 animals for each reassortant, 14 for T1, and 9 for T3.
[d] Location in the virion of the polypeptide encoded by the corresponding genome segment.

specific activity in lung tissue, the reassortant viruses were clearly divided into two groups (Table 2). One group (T3, 1HA3, EB73, EB138, EB143, E3, and G2) exhibited a specific activity in lungs of <6 cpm/mg of lung tissue, whereas the other group (T1, 3HA1, H24, EB1, EB93, EB98, EB126, and EB144) was characterized by a specific activity in lungs of >60 cpm/mg of lung tissue. All reassortants taken up efficiently by the lungs (>60 cpm/mg of tissue) contained the S1 gene from T1 (Table 2), whereas all reassortants with low lung clearance (<6 cpm/mg tissue) contained the S1 gene from T3. Importantly, no other viral gene was found to segregate in a similar way. These results indicate that the S1 gene of T1 is responsible for the difference in lung uptake between reovirus T1 and T3.

Clearance of reovirus T1 ISVPs. To determine whether the σ1 protein was not only necessary but sufficient for uptake of T1 by the lungs, we analyzed the effect of removing part of the outer capsid on the clearance of T1. ^{35}S-labeled T1 was subjected to limited proteolytic digestion in vitro with chymotrypsin. This treatment results in total removal of the σ3 protein and cleavage of μ1c to δ (26) (Fig. 2A). Chymotrypsin-treated virus (ISVPs) was injected intravenously, and the clearance properties were compared with those of intact ^{35}S-labeled T1 5 min after the injection. Partial digestion of the outer capsid of T1 did not affect the ability of the virus to be cleared by the lungs. In fact, the treated virus was cleared more efficiently by the lungs than was undigested T1 (Fig. 2B). The radioactivity content of the liver was proportionately decreased from 33 ± 3 cpm/mg for T1 to 6 ± 1 cpm/mg for T1 ISVPs. There was no significant change in the amount of radioactivity observed in the blood whether the virus was or was not treated with chymotrypsin (Fig. 2B).

Effect of a monoclonal anti-σ1 antibody on clearance of T1 and T1 ISVPs. To define the role of the σ1 protein in the uptake of T1 by the lungs further, the effect of a monoclonal antibody directed against the T1 σ1 protein was analyzed. T1 or T1 ISVPs were incubated overnight in the presence of the neutralizing antibody 5C6 and injected intravenously. The rats were killed after 20 min, and the organs were processed for radioactivity measurement. Incubation of T1 with the antibody before injection resulted in 98% inhibition of uptake by the lungs (Fig. 3). A proportional increase in liver and spleen radioactivity content was observed (Fig. 3), and blood radioactivity was similar in both cases (data not shown). A similar inhibition of lung uptake was observed when the monovalent Fab fragment of the 5C6 antibody was used instead of the intact antibody (Fig. 3). In addition, uptake of T1 ISVPs by the lungs was also inhibited by the intact 5C6 antibody (Fig. 2B) or its Fab fragment (data not shown). Incubation of T1 with a monoclonal antibody directed against the T3 σ1 protein (G5) did not affect the pattern of uptake of T1 (Fig. 3) or T1 ISVPs (data not shown).

Clearance of a T1 variant in the σ1 protein. To see whether mutations in the σ1 protein affected the pattern of viral clearance, we examined a variant virus carrying a lesion in the σ1 protein (B9B). The pattern of uptake of B9B after intravenous injection was markedly different from that of parental T1 (Table 3). At 20 min after injection, specific activity in the lungs was 20% of that of control animals that had received an injection of T1. However, the virus was taken up efficiently by the liver and spleen, and a low specific activity (3.1 ± 0.2 cpm/mg) in blood was measured (Table 3). These data indicate that a site on the T1 hemagglutinin is important for T1 uptake by the lungs.

DISCUSSION

To understand how viruses interact with a host after their entry into the bloodstream, we analyzed the clearance of reovirus from the bloodstream after intravenous injection. Both reovirus T1 and T3 were cleared rapidly from the circulation, as previously reported (30). However, despite similar sizes and outer capsid organization, reovirus T1 and T3 were cleared differently; reovirus T1 is taken up mainly by the lungs and the liver, whereas T3 is taken up predom-

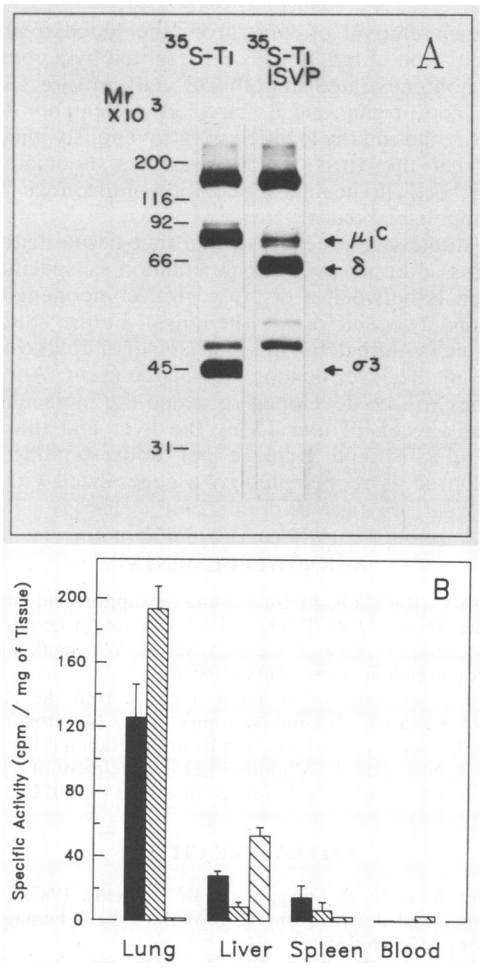

FIG. 2. Clearance of reovirus T1 and T1 ISVPs by the lungs. (A) Purified ^{35}S-labeled T1 was digested with chymotrypsin, repurified on a CsCl gradient, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions. The gel was dried and processed for autoradiography. Molecular weight standards (Bio-Rad Laboratories, Richmond, Calif.) were separated on the same gel and used for molecular weight determination. (B) ^{35}S-labeled T1 (black), ^{35}S-labeled T1 ISVPs (narrow hatched), and ^{35}S-labeled T1 ISVPs incubated with Fab fragments of 5C6 overnight (wide hatched) were injected intravenously; 5 min later, the animals were killed and organ-associated radioactivity was determined. The results are presented as specific activities (mean ± 1 standard deviation; n = 4).

inantly by the liver and very little virus is taken up by the lungs. Since T1 and T3 were cleared from the bloodstream within 10 min after injection, we postulated that a component(s) on the virus surface must be involved in mediating the specific clearance. By using intertypic reassortants and assaying the different patterns of clearance of T1 and T3, we determined that the T1 S1 gene is responsible for this difference. This gene encodes two proteins, the σ1 protein (the viral hemagglutinin) and a small nonstructural polypeptide of unknown function, σ1NS (2, 16). Since σ1 is on the viral particle surface and since σ1NS is not associated with reovirus particles (6, 8, 9, 20), we believe that the σ1 protein is the protein responsible for the difference in lung uptake between T1 and T3.

The genetic analysis indicated that the T1 hemagglutinin is a critical determinant and is responsible for the different patterns of uptake of T1 and T3 by the lungs. These data do

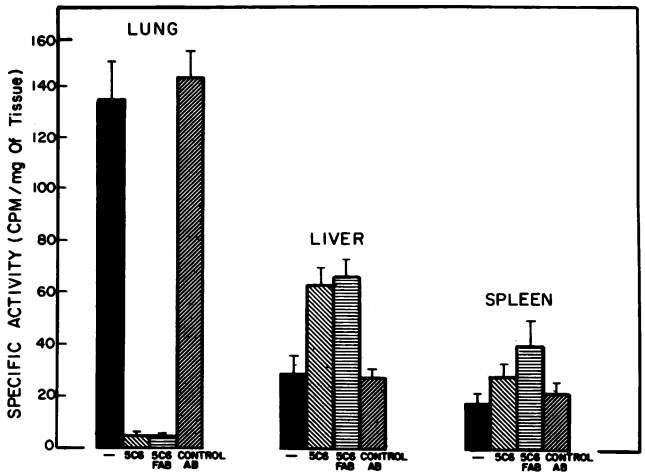

FIG. 3. Effect of T1 σ1-specific monoclonal antibody on clearance of reovirus T1. ^{35}S-labeled T1 was incubated with 5C6, a T1 σ1-specific monoclonal antibody, Fab fragments of 5C6, or a control monoclonal antibody directed against the σ1 protein of T3 overnight. The virus and antibody were injected intravenously into rats, and 20 min later the animals were killed and organ-associated radioactivity was determined. The results are presented as specific activities (mean ± 1 standard deviation; n = 5).

not indicate whether the σ1 protein is sufficient for uptake of T1 by the lungs. We used other approaches to determine whether any of the other outer capsid proteins were involved in uptake of T1 by the lungs. We observed that uptake by the lungs of T1 digested with chymotrypsin (T1 ISVPs), which lacks the outer capsid protein σ3 and contains a cleaved μ1C, was not decreased. In fact, a significant increase in uptake by the lungs was observed after chymotrypsin digestion. The mechanism underlying this increased uptake is not clear; however, treatment of T1 with chymotrypsin results in extension of σ1 from the outer capsid of the virion (7). Extension of the σ1 protein from the virion could make a binding site on that protein more exposed, more accessible, or both, thereby facilitating the removal of T1 ISVPs from the bloodstream. These results demonstrate that uptake of T1 by the lungs is not dependent on the σ3 protein or on an intact μ1C and thus confirm the preponderant role played by the T1 σ1 protein. Alternatively, the presence of the σ3 protein on the particle surface could diminish uptake of both viruses by the lungs independently of the extension of the σ1 protein.

Treatment of T1 with intact or Fab fragment of a T1 anti-σ1 monoclonal antibody completely inhibited uptake of

TABLE 3. Clearance of ^{35}S-labeled T1 and ^{35}S-labeled B9B by various organs

Organ	Mean (±1 SD) sp act in lungs (cpm/mg of tissue)[a]	
	T1	B9B
Lung	166 ± 34	32 ± 13
Liver	29 ± 4	58 ± 6
Spleen	22 ± 9	43 ± 23
Kidney	2 ± 0	4 ± 2
Brain	<1	3 ± 0
Blood	1 ± 0	3 ± 0

[a] Specific activities for ^{35}S-labeled T1 and ^{35}S-labeled B9B are from five animals each.

T1 by the lungs. When the intact 5C6 antibody was used, the inhibition of lung uptake may have been due to viral aggregation or blocking of a specific binding site on the σ1 protein. The increased liver and spleen uptake observed after injection of virus incubated with the intact antibody may have been due to either the presence of Fc receptor found in these tissues or aggregation of the virus, resulting in larger circulating particles, or it may simply have been a consequence of the larger amount of circulating virus reaching those organs because of the inhibition of uptake by the lungs. The results with Fab indicate that inhibition of uptake by the lungs can occur independently of viral aggregation. These data imply that Fab binding to the hemagglutinin, either at a specific binding site or by steric hindrance of a binding site, is capable of inhibiting uptake of T1 by the lungs. The increase in uptake by the liver and spleen does not appear to be mediated through the Fc fragment, since this increase was also observed when the Fab fragment of the antibody was used. Additionally, a mutant in the T1 σ1 protein exhibited decreased ability to be taken up by the lungs. This potentially identifies a site on the hemagglutinin responsible for uptake by the lungs. From these experiments, we conclude that the σ1 protein is responsible for uptake of T1 by the lungs.

The σ1 protein serves as the cell attachment protein (13) as well as the viral protein that mediates hemagglutination (31). These two binding functions appear to be separable on the T3 σ1 protein (24). It is unknown whether these two binding activities are in distinct or overlapping regions of the T1 σ1 protein. The S1 gene, which encodes the σ1 protein, is also responsible for determining the pathway of spread to the central nervous system in neonatal mice after intramuscular inoculation (29); reovirus T1 (Lang) spreads hematogenously, and T3 (Dearing) spreads neurally. Additionally, the T1 S1 gene determines the ability of reovirus to spread to the spleen after peroral inoculation (12). We believe that the ability of the σ1 protein to bind to specific cellular receptors present in the lung microcirculation, possibly on endothelial cells, mediates the specific uptake of T1 by the lungs.

Intravenous inoculation of a virus and clearance from the bloodstream may be analogous to a primary viremia in which virus first enters the bloodstream and then localizes to specific tissues, mainly the reticuloendothelial system (e.g., liver and spleen). Viruses could potentially use the bloodstream as a pathway to spread to target tissues; however, the fate of viruses in target organs is critical in determining the efficiency of this spreading strategy. The presence or absence of specific cells able to support viral replication within a target organ will determine the fate of viruses after their uptake by that organ. Clearance of a virus from the bloodstream and uptake by a target organ can, therefore, be considered as one aspect of the hematogenous pathway of spread within a host.

Differences in clearance of viruses are thought to play a role in virus-host interaction. Apathogenic strains of Venezuelan equine encephalomyelitis virus are cleared more rapidly from the bloodstream after intravenous inoculation than are pathogenic strains (10). Additionally, apathogenic strains of Sindbis virus or Sindbis virus treated with neuraminidase are also removed from the bloodstream more rapidly than is pathogenic or untreated virus, respectively (18, 21). Therefore, ability to remain in the bloodstream has been correlated with the ability of these viruses to initiate an infection in vivo.

Uptake of viral particles by the lungs is of particular interest, since that organ has not been previously identified as a site of removal of virus from the bloodstream. The microcirculation of the lungs is represented by a continuous layer of nonfenestrated endothelial cells in direct contact with the bloodstream and its circulating elements (4). Preliminary findings on the location of intravenously injected T1 indicate that the virus rapidly becomes associated with endothelial cells in the microcirculation of the lungs (Warner et al., unpublished data).

These studies have demonstrated that tissue distribution of reovirus after intravenous inoculation is specific. This interaction is between a discrete viral component (the σ1 protein) and a specific organ (the lungs). Future studies will try to define in more detail the mechanism of uptake of T1 by the lungs at the level of lung microcirculation. Alternative approaches will be developed to define the molecular basis for the uptake of T1 and T3 by the liver and the spleen. These studies should increase our understanding of the multistep process represented by a generalized viral infection.

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