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Mechanisms of Monoclonal Antibody-Mediated Protection Against Virulent Semliki Forest Virus

WIL A. M. BOERE,* BARRY J. BENAISSA-TROUW, THEO HARMSEN, TINEKE ERICH, CORNELIS A. KRAAIJEVELD, AND HARM SNIPPE

Laboratory of Microbiology, Faculty of Medicine, State University of Utrecht, 3511 GG Utrecht, The Netherlands

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Both neutralizing and nonneutralizing immunoglobulin G2a monoclonal antibodies (MAs) directed against the E_2 glycoprotein of Semliki Forest virus (SFV) protected mice prophylactically and therapeutically against virulent SFV infection. The neutralizing MAs, however, conferred protection to mice at lower doses than did nonneutralizing MAs. The antibody-dependent, complement-mediated cytolysis of SFV-infected L cells was effectuated by both kinds of antibodies, but again neutralizing MAs were more effective. Removal of the Fc part of the neutralizing MA UM 5.1 by pepsin digestion resulted in a 100-fold reduction of the neutralization titer (10^4 versus 10^6) and a complete loss of its capacity to mediate antibody-dependent, complement-mediated cytolysis. Passive protection of infected mice occurred only after administration of relatively high doses of $F(ab')_2$ of MA UM 5.1 (30.0 μg versus 0.1 μg). $F(ab')_2$ fragments prepared from the nonneutralizing MA UM 4.2 had lost their protective capacity completely. Surprisingly, the nonneutralizing MA UM 4.2 retarded virus growth in mouse fibroblasts (L cells), although inhibition was at much higher doses than with the neutralizing MA UM 5.1. Furthermore, both MAs promoted the uptake of virulent SFV in the Fc receptor-bearing WEHI-3 cells. The results suggest that nonneutralizing MAs protect mice not only by antibody-dependent, complement-mediated cytolysis but also by growth inhibition and enhanced uptake of SFV in the nonpermissive macrophages of BALB/c mice. This hypothesis is supported by the absence of viremia in recipients of nonneutralizing MA UM 4.2 at 24 h after infection.

Previous studies have indicated that both neutralizing and nonneutralizing monoclonal antibodies (MAs) directed against either the E_1 or E_2 glycoprotein of Semliki Forest virus (SFV) protect mice passively against an otherwise lethal infection with virulent SFV (1, 2). Nonneutralizing MAs could be subclassified as protective and nonprotective MAs. The protective capacity of MAs is presumably related to the epitope recognized and the immunoglobulin subclass (2, 7, 17, 18). In earlier studies, mice were infected 2 h after the intravenous transfer of MAs. In the present study, we demonstrate that both neutralizing and nonneutralizing MAs can be successfully used for long-term prophylaxis and short-term therapy. This study investigates the mechanism(s) by which nonneutralizing MAs could prevent lethal encephalitis caused by virulent SFV in mice. One of the mechanisms by which nonneutralizing MAs may provide protection is antibody-dependent, complement-mediated cytolysis (ADCMC) which already has been suggested for the closely related Sindbis virus (21). We also considered and investigated other possible mechanisms for these nonneutralizing MAs, including in vivo neutralization, retardation of virus replication by blocking of virus receptors, and MA-mediated uptake of SFV in nonpermissive macrophages.

In this study, the biological functions of E_2-specific MAs of the immunoglobulin G_{2a} (IgG_{2a}) immunoglobulin subclass and their $F(ab')_2$ fragments were compared.

MATERIALS AND METHODS

Virus strains. The virulent strain SF/LS 10 C1/A was received from C. J. Bradish (3) and passed twice through BALB/c mice. Brain suspensions of the second passage were pooled, divided in small portions, and kept in ampoules above liquid nitrogen. The repeated plaque titration of thawed brain material resulted in virus titers of 6×10^5 PFU/ml. Brain suspensions containing virulent SFV were used for plaque reduction tests and infection of mice. The intraperitoneal (i.p.) 50% lethal dose (LD_{50}) for male BALB/c mice was 1 to 2 PFU of the virulent strain. The avirulent SFV strain MRS MP 192/7 was obtained from K. G. Oei (Royal Tropical Institute of Amsterdam, Amsterdam, The Netherlands). The general virological methods have been described previously (2, 14).

Cells and media. L cells, a continuous line of mouse fibroblasts grown in Dulbecco minimal essential medium with 0.01 M *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid supplemented with 5% calf serum and antibiotics, were used throughout this study. The complete growth medium was also used for diluting infectious virus or cells to the required concentrations. The same medium was used for WEHI-3 cells, which have characteristics of macrophages (23).

Animals. Inbred BALB/c mice were bred and maintained in our own animal house. Male mice of ca. 12 weeks of age were used for protection experiments against i.p. infection with virulent SFV.

Monoclonal antibodies. The production, purification, and biological characterization of the IgG_{2a} MAs directed against the E_2 glycoprotein of SFV are described in other papers (1, 2).

Preparation of $F(ab')_2$ fragments. $F(ab')_2$ fragments of MAs UM 4.2 and UM 5.1 were prepared by pepsin digestion. The optimal conditions for pepsin digestion were determined by titrating the pepsin (no. P-7012; Sigma Chemical Co., St. Louis, Mo.) in an analytical experiment, after which $F(ab')_2$ production was tested by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 4 to 30% gradient gel. Both MAs UM 4.2 and UM 5.1 were of the IgG_{2a} subclass but differed in sensitivity to pepsin. For UM

* Corresponding author.

4.2, a pepsin-to-antibody ratio on the protein base of 1:10 was necessary; for UM 5.1, a 1:40 ratio was suitable. Digestion was performed for 24 h at 37°C in 0.1 M sodium acetate buffer (pH 4.8).

The $F(ab')_2$ fragments were isolated by gel filtration with a column of Bio-Gel P150 (100- to 200-mesh; Bio-Rad Laboratories, Richmond, Calif.) and elution with 0.1 M Tris-hydrochloride buffer (pH 7.7) containing 0.2 M NaCl and 0.02 M EDTA. To remove possibly undigested antibodies, the eluate containing the $F(ab')_2$ fragments was adjusted to pH 8 and subsequently passed over a protein A-Sepharose column (Pharmacia, Uppsala, Sweden) (8). The eluent was tested for purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and was free of Fc fragments or nondigested IgG_{2a} molecules. Protein content was estimated by the Lowry method (15).

To confirm that the $F(ab')_2$ fragments had retained their binding capacity to the virus, these fragments were coupled to horseradish peroxidase and subsequently tested in a direct enzyme-linked immunosorbent assay against purified avirulent SFV. The absorbance values measured were comparable to those of horseradish peroxidase-conjugated complete MAs. This indicated that the antibody-binding site was not destroyed by the pepsin digestion.

ADCMC. ADCMC of SFV-infected cells, by mutual action of E_2-specific MA and guinea pig complement, was measured as described by King et al. (12). Briefly, 3×10^6 L cells were labeled with 100 μCi of $Na_2{}^{51}CrO_4$ (specific activity, 350 to 600 mCi/mg of chromium; Radiochemical Centre, Amersham, England). After incubation for 1 h at 37°C, the cells were washed three times, infected with either virulent or avirulent SFV at a multiplicity of infection of 10, and seeded in 96-well plates at a concentration of 3×10^4 cells per well. Six hours after infection, fivefold dilutions of purified MA (starting with 400 μg per well) and a 1:15 dilution of guinea pig complement were added. At 8 h postinfection, triplicate samples of the supernatant fluids were analyzed for ^{51}Cr release in a Philips 4800 gamma counter (Philips Almelo, The Netherlands). Wells receiving medium, complement, inactivated complement (30 min at 56°C) plus MAs, or 1% Triton X-100 served as controls. The latter served as the control for the 100% value of released radioactivity. ADCMC titers were expressed as percentages of specific ^{51}Cr release.

Determination of MA-mediated retardation of SFV growth in cell culture with horseradish peroxidase-labeled MA. Tenfold dilutions of purified MAs were added to either a suspension of L cells (10^6 cells per ml) or WEHI-3 cells (10^6 cells per ml) and incubated for 30 min at 37°C. Control cell suspensions were not mixed with MAs. The cell suspensions were pipetted in 0.05-ml volumes (5×10^4 cells) into wells of flat-bottomed, 96-well plates (catalog no. 3596; Costar Plastics, Cambridge, Mass.) already containing virulent SFV in 0.05 ml of complete growth medium per well. The standard virus inoculum was 1,500 PFU per well, corresponding to a multiplicity of infection of ca. 0.03. The plates with infected cells were incubated at 37°C. After selected time intervals (8, 12, 16, and 20 h), the supernatant fluids were discarded, and the monolayers of either L cells or WEHI-3 cells were fixed by the addition of 0.05% glutaraldehyde in phosphate-buffered saline (PBS; E. Merck, AG, Darmstadt, Federal Republic of Germany) and incubated for 10 min at 37°C. The plates were washed with tap water, rinsed once with PBS (pH 7.2), and finally shaken dry. At the indicated time intervals (one multiplication cycle takes ca. 8 h), virus antigen was detected by incubation with a mixture of two horseradish peroxidase-conjugated (1:5,000 dilution) E_1-specific MAs (UM 8.47 and UM 8.64) (22). After incubation for 1 h at 37°C, the plates were washed three times with PBS and shaken dry. The amount of bound enzyme was visualized by incubating the wells with 0.05 ml of substrate solution containing 3',3',5',5'-tetramethylbenzidine (Sigma) and urea peroxide (Organon Teknika, Boxtel, The Netherlands) (22). After 30 min of incubation at room temperature, the enzyme reaction was stopped with 0.05 ml of 1 M H_2SO_4 per well, and peroxidase activity was quantified by measuring the optical density at 450 nm with a Titertek Multiscan instrument (Flow Laboratories, Irvine, Scotland). The absorbance values shown are the means of duplicates.

TABLE 1. Biological characteristics of various IgG_{2a} MAs with specificity for the E_2 glycoprotein of SFV

MA	Antigenic determinant[a]	Reciprocal of neutralization titer[b]	% Lysis of virulent SFV-infected L cells in ADCMC[c]	In vivo minimal protective dose (μg)[d]
UM 8.130	$E_2{}^a$	<1	11.5	100.0
UM 4.2	$E_2{}^a$	<1	11.9	10.0
UM 8.55	$E_2{}^c$	10^3	20.6	10.0
UM 8.48	$E_2{}^c$	10^3	26.1	10.0
UM 8.79	E_2	10^4	26.7	1.0
UM 5.1	$E_2{}^d$	10^6	43.9	0.1

[a] Results are as determined previously (2).

[b] The highest dilutions of purified monoclonal antibodies (1 mg/ml) causing 50% plaque reduction with virulent SFV.

[c] The test was performed with a 1:50 dilution (80 μg per well) of purified MAs. Less than 5% lysis was measured in controls of infected cells with MAs and inactivated complement.

[d] Full protection of mice ($n = 6$) against i.p. challenge with 10 LD_{50} units of virulent SFV 2 h after intravenous transfer of purified MAs.

Passive protection by MA. Protection provided by MAs in BALB/c mice against lethal encephalitis caused by virulent SFV was determined after intravenous injection of 0.2-ml volumes of serial dilutions in PBS of purified MAs. At various intervals before or after passive transfer of MAs, groups of five to eight mice were injected i.p. with 10 LD_{50} units (16 PFU) of SFV. To quantitate protection, mice were observed for 21 days. Control mice that received PBS instead of MAs generally died within 6 days after challenge.

Determination of virus titers in blood and peritoneal cavity. For the determination of viremia, blood was collected by retro-orbital puncture. Peritoneal contents were obtained after the injection of 2 ml of Dulbecco minimal essential medium into the peritoneal cavity. After slight massage, the animal was killed, the abdomen was opened, and the fluid was collected with a pipette (1.2 to 1.8 ml). After centrifugation, the supernatant was used for virus titration if necessary by inoculating the total harvest on monolayers of L cells in 16-mm wells of plastic 24-well plates (Costar).

RESULTS

Prophylaxis and therapy of lethal encephalitis with E_2-specific MAs. Neutralization titers of protein A-purified E_2-specific MAs of immunoglobulin subclass IgG_{2a} were determined against virulent SFV. The minimal fully protective doses of protein A-purified MAs were assessed by injecting groups of mice intravenously with MAs followed 2 h later by i.p. injection of 10 LD_{50} units of virulent SFV (Table 1). Although neutralizing and nonneutralizing MAs afforded protection, the results indicated an inverse relationship between the protective dose and neutralization titer. MAs with the highest neutralization titers protected mice

TABLE 2. Prophylaxis and therapy of lethal SFV infection in mice with either nonneutralizing MA UM 4.2 or neutralizing MA UM 5.1[a]

MA transferred	Interval between treatment and challenge (days)	No. survivors/no. injected with MA dose (μg) of:			
		100	10	1	0.1
UM 4.2	−112	1/5	1/6	—	—
UM 5.1		—	3/6	2/5	0/6
UM 4.2	−28	6/6	0/6	—	—
UM 5.1		—	6/6	6/6	1/6
UM 4.2	−7	6/6	2/6	—	—
UM 5.1		—	6/6	6/6	6/6
UM 4.2	+1	6/6	—	—	—
UM 5.1		—	5/6	5/6	—
UM 5.1	+2	4/6	—	—	—
UM 5.1	+2 and +3	6/6	—	—	—

[a] Graded doses of purified MAs were transferred intravenously 112, 28, or 7 days before or 1, 2, or 2 and 3 days after i.p. challenge with 10 LD_{50} units of virulent SFV. Control mice received PBS only, and 29 of 30 died 5 to 6 days after injection (data not shown). —, Not tested.

with the lowest quantities of purified MA. Consistent with these results were the observations made in long-term prophylaxis and short-term therapy by MA UM 4.2 and MA UM 5.1 (Table 2). In prophylaxis, a dose of 100 μg of the nonneutralizing MA UM 4.2 afforded complete protection when the MAs were injected 4 weeks before infection; only 1 μg of the neutralizing MA UM 5.1 was required. Therapy of mice with progressive lethal encephalitis due to SFV infection was complicated by the short mean survival time of these mice (6 days). Nevertheless, such infected mice were effectively therapeutically treated with either neutralizing or nonneutralizing MAs when administered 24 h after infection. For example, all mice were cured when a dose of 100 μg of MA UM 5.1 was given twice at 48 and 72 h after infection. The results showed clearly that the capacity to neutralize infectious virus in vivo was an important factor in MA-mediated protection. On the other hand, the protection of mice afforded by relatively high doses of nonneutralizing MAs indicated that other mechanisms were involved.

Growth inhibition of virulent SFV in cell culture by neutralizing and nonneutralizing MAs. Both the neutralizing and nonneutralizing MAs induced lysis of L cells in the presence of complement (Table 1). The nonneutralizing MAs were, however, considerably less effective than the neutralizing MAs. Besides ADCMC of infected cells, other mechanisms might be involved in the protection afforded by nonneutralizing MAs in mice after infection with virulent SFV. One of these might be the promotion of virus uptake by Fc receptor-bearing phagocytes. Because SFV did not replicate in macrophages of BALB/c mice (13), enhanced uptake of virus by those phagocytes delayed the progression of infection.

For this reason, growth inhibition of SFV by MAs was studied in vitro in L cells (mouse fibroblasts) and cell line WEHI-3 (mouse macrophages; Fc receptors present, but a permissive host). Peritoneal macrophages of BALB/c mice served as the control. The MAs were added in graded concentrations similarly to the in vivo protective doses. Both neutralizing MA UM 5.1 and nonneutralizing MA UM 4.2 retarded the growth of SFV in L cells (Fig. 1). Due to its neutralizing capacity, however, the effectiveness of MA UM 5.1 to inhibit virus growth in L cells was 400 times greater than that of MA UM 4.2. Doses of MAs UM 4.2 and UM 5.1

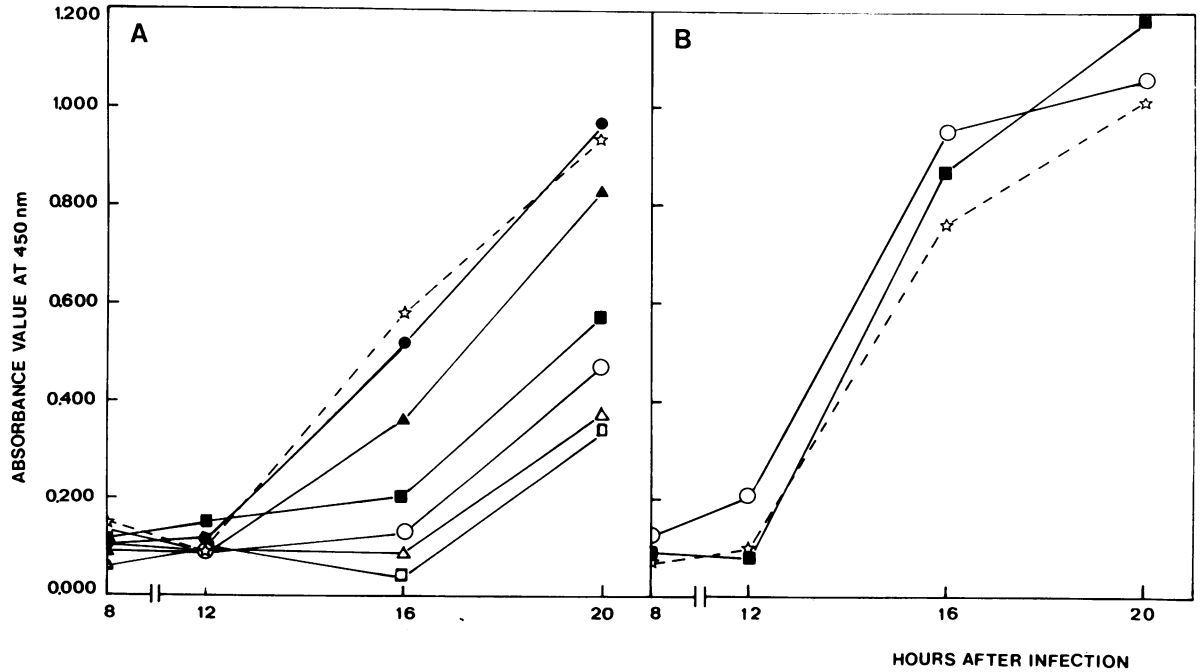

FIG. 1. The effect of neutralizing MA UM 5.1 and nonneutralizing MA UM 4.2 on the growth of virulent SFV in L cells and WEHI-3 cells. Suspensions of cells, mixed with various concentrations of MAs, were seeded in 96-well plates which already contained a virus inoculum of 1,500 PFU per well. At various time intervals after infection (8, 12, 16, and 20 h), the monolayers of cells were fixed. The monolayers of either L cells (A) or WEHI-3 cells (B) were incubated with a mixture of horseradish peroxidase-labeled anti-E_1 MAs. Absorbance values at 450 nm are presented for the following concentrations of UM 5.1 and UM 4.2 per 0.1 ml; 0.025 μg (○), 0.25 μg (△), and 2.5 μg (□), for UM 5.1; and 0.1 μg (●), 1.0 μg (▲), and 10 μg (■) for UM 4.2. ☆, Infected cells not mixed with MAs which served as a control.

TABLE 3. Efficacy of MAs UM 4.2 and UM 5.1 and their $F(ab')_2$ fragments in virus neutralization and protection

MA	Reciprocal of neutralization titer[a]	% Lysis of avirulent SFV-infected L cells in ADCMC[b]	In vivo protection[c] dose of MA (μg)					
			100	30	10	3	0.3	0.1
UM 4.2 intact molecule	<1	6	6/6	—	6/6	—	—	—
UM 4.2 $F(ab')_2$ fragment	<1	<3	0/6	—	0/6	—	—	—
UM 5.1 intact molecule	10^6	33	—	—	—	6/6	6/6	6/6
UM 5.1 $F(ab')_2$ fragment	10^4	<3	—	4/5	1/5	1/5	1/8	0/8

[a] The highest dilution of a purified monoclonal antibody (1 mg/ml) causing 50% plaque reduction.
[b] ADCMC was performed with 50 μg per well of purified MAs.
[c] Number of survivors versus number injected. —, Not tested.

which retarded growth in L cells were not inhibitory in WEHI-3 cells. This might be caused by the promotion of virus uptake by MAs and Fc receptors on the WEHI-3 cells. In peritoneal macrophages, no virus replication could be detected.

Effects of removal of Fc parts from MAs on neutralization characteristics. Highly purified $F(ab')_2$ fragments of both neutralizing MA UM 5.1 and nonneutralizing MA UM 4.2 were compared with their parent molecules with regard to neutralization, ADCMC, and protection (Table 3). On equivalent weight bases, the neutralization titer of MA UM 5.1 diminished 100-fold after the removal of its Fc fragment. In the ADCMC test, lysis of avirulent SFV-infected L cells by these molecules was annihilated. These effects are reflected in a diminished efficacy of the $F(ab')_2$ fragments to protect mice against a challenge with virulent virus.

Removal of the Fc fragment of the nonneutralizing MA UM 4.2 abolished the already low effectivity in ADCMC, and there was no in vivo protection at all with the highest tested dose (100 μg; Table 3).

Effect of neutralizing and nonneutralizing MAs on virus titers in blood stream and peritoneal cavity of mice infected with virulent SFV. The clearance of circulating virulent virus by the neutralizing MA UM 5.1 and the nonneutralizing MA UM 4.2 was studied in vivo. Purified MA was injected intravenously 2 h before i.p. infection with either 160 PFU (100 LD_{50} units) or 404 PFU (252 LD_{50} units) of virulent SFV. The numbers of circulating viral particles in blood and peritoneal cavities of four individual mice were determined 45 min and 24 h after infection (Table 4). In control mice, the virus spread to the blood before virus multiplication had occurred (45 min). In mice which received 50 μg of the nonneutralizing MA UM 4.2, no effect on viral distribution and actual viral counts was observed after 45 min. In contrast to control mice, however, these mice did not develop a viremia 24 h later. Administration of either 50 or 0.5 μg of neutralizing MA UM 5.1 resulted in the disappearance of infectious virus from the circulation at both time intervals tested (45 min and 24 h), whereas no viremia developed and all mice survived. Removal of the Fc fragment of MA UM 5.1 resulted in a disabled clearance efficacy of infectious virus. Although there was a reducing effect of $F(ab')_2$ fragments on viremia at both 45 min (no virus detected) and 24 h (20-fold lower compared with control mice), the reduction was not to such a degree that death due to encephalitis was prevented as indicated by the absence of survivors in an accompanying group of mice.

DISCUSSION

Previous studies (1, 2) have indicated that both neutralizing and nonneutralizing MAs directed against either the E_1 or E_2 glycoproteins of SFV could confer protection to mice against the normally lethal brain damage caused by the in vivo replication of virulent SFV. However, in these earlier studies the MAs were injected a mere 2 h before challenge with infectious virus. In the present study, we demonstrate that both neutralizing and nonneutralizing MAs also assure complete survival in long-term prophylaxis (administration of MAs 28 days before infection) and in short-term therapy (administration of MAs 24 h after infection). With the

TABLE 4. The effect of neutralizing MA UM 5.1 and non-neutralizing MA UM 4.2 on virus titers in vivo

Treatment of mice[a] with MA			Interval between infection and collection of body fluids (min)	Infectious dose of virulent SFV[b] (PFU)	Mean ± SD of virus recovered (PFU/ml) from:		No. of survivors/ no. injected
Clone no.	Pepsin digestion	Dose (μg)			Serum	Peritioneal cavity	
PBS		0	45	160	3 ± 2	28 ± 10	NT[c]
PBS		0	1,440	32	530 ± 264	10,715 ± 4,424	
UM 4.2	−	50	45	160	6 ± 3	18 ± 4	NT
UM 4.2	−	50	1,440	32	0	1 ± 1	
UM 5.1	−	50	45	160	0	0	NT
UM 5.1	−	50	1,440	32	0	0	
PBS		0	45	404	48 ± 16	127 ± 65	0/4
PBS		0	1,440	70	10,380 ± 5,077	16,950 ± 9,742	
UM 5.1	−	0.5	45	404	0	38 ± 37	4/4
UM 5.1	−	0.5	1,440	70	0	7 ± 6	
UM 5.1	+	0.5	45	404	0	97 ± 24	0/4
UM 5.1	+	0.5	1,440	70	545 ± 399	279 ± 184	

[a] Mice (n = 4) were injected intravenously with purified MA in 0.1 ml of PBS (pH 7.2).
[b] Mice were injected i.p. with diluted virulent SFV in 0.5 ml of PBS.
[c] NT, Not tested.

superior neutralizing MA UM 5.1, it is even possible to start effective therapy 48 h after infection of mice, whereas untreated animals die after ca. 6 days. The long-term protection may be due to a prolonged half-life time of the MAs of IgG_{2a} subclass. The half-life time of mouse IgG_{2a} myeloma proteins in serum is ca. 5 days (10). The half-life times of MAs in extravascular fluids (e.g., liquor) or attached to tissue macrophages are not known yet but may still contribute to the observed protection in long-term prophylaxis. Another factor which may confer protection is formation of antiidiotypic antibodies (20).

On an equal weight base, the neutralizing MAs were more effective in protection than nonneutralizing MAs. Moreover, the protective dose of purified neutralizing MAs was clearly inversely related to their neutralization titer. The mechanism of virus neutralization is still not completely understood (6). Hitherto one could only speculate on the mechanism by which nonneutralizing MAs afford protection. For virulent alphaviruses, ADCMC of infected cells is proposed (21). Early lysis of infected cells leads to reduced production of virulent virus, which allows the animal to build up its own effective immune response before irreversible damage has been inflicted. Surprisingly, the effectiveness of the MAs in the ADCMC test was correlated to their neutralization titer. The nonneutralizing MAs UM 8.130 and UM 4.2 induced only modest complement-mediated lysis of L cells infected with virulent SFV. In contrast, the excellent neutralizing MAs UM 8.79 and UM 5.1 were highly effective in the ADCMC test. Therefore, we reasoned that, besides ADCMC, both nonneutralizing and neutralizing MAs also afforded protection by other mechanisms.

First, we demonstrated that the replication of virulent SFV in vitro is inhibited in the presence of the nonneutralizing MA UM 4.2. Inhibition of virus growth occurred at a dose similar to that which is needed to render mice immune to SFV. We conjecture that a mere blocking of effective contacts of nonneutralizing MAs between virus and its cell receptor results in a diminished viremia in infected mice. A second mechanism might be the clearance of virus-antibody complexes in nonpermissive macrophages. Various investigators have shown that MAs enhance the uptake of virus in Fc receptor-bearing macrophages (4, 11, 19). We observed that recipients of 50 μg of nonneutralizing MA UM 4.2 did not develop a viremia (see virus recovery at 24 h after infection [Table 4]). Although MA UM 4.2 is nonneutralizing in vivo, as indicated by the presence of infectious virus in blood at 45 min of infection, it is very likely that these antibodies promote the clearance of virulent SFV by nonpermissive macrophages. The latter will be the subject of further study. It is of interest that the activation of macrophages (which occurs during viral infection) leads to an increased number of IgG_{2a} receptors on macrophages (9); therefore, the MAs we used (only IgG_{2a} subclass) have an advantage over MAs of other IgG subclasses. This is further supported by data presented in a previous report (2) in which we observed that two IgG_1 MAs (nonneutralizing SFV) did not show any protective capacity. The idea of an enhanced clearance of virulent SFV by nonpermissive macrophages is further supported by in vitro experiments. Inhibiting doses for replication in L cells of both nonneutralizing MA UM 4.2 and neutralizing MA UM 5.1 are not inhibitory in the continuous line of Fc receptor-bearing WEHI-3 cells, which have a macrophage ancestry (23).

The role of the Fc part of MAs in protective immunity is further exemplified in experiments in which this part was removed by pepsin digestion. $F(ab')_2$ fragments of the nonneutralizing MA UM 4.2 lost their protective capacity in vivo completely, whereas at least a 100-fold reduction was observed for $F(ab')_2$ fragments obtained from the neutralizing MA UM 5.1. This loss in effectiveness of biological functions might be due to reduced uptake by macrophages and the absence of a mechanism in vivo which is similar to ADCMC. Moreover, $F(ab')_2$ fragments of the neutralizing MA UM 5.1 were, on an equal basis, 100-fold less effective in neutralization (10^4 to 10^6) compared with parent molecules. We speculate that these $F(ab')_2$ fragments are less able to deform virus particles which is assumed to play a role in neutralization (5, 6, 16).

In conclusion, neutralizing MAs protect mice more efficiently against infection with virulent SFV than do nonneutralizing MAs. Both neutralizing and nonneutralizing MAs show the inhibition of virus multiplication in L cells, but less (neutralizing MA UM 5.1) or no (nonneutralizing MA UM 4.2) inhibition is observed in Fc receptor-bearing cells. The Fc part in both neutralizing and nonneutralizing MAs plays an important role in the mediation of protection and ADCMC of infected cells.

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