JOURNAL OF VIROLOGY, May 1988, p. 1803–1807
0022-538X/88/051803-05$02.00/0
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Vol. 62, No. 5

Clearance of Lymphocytic Choriomeningitis Virus in Antibody- and B-Cell-Deprived Mice

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Received 21 September 1987/Accepted 10 February 1988

The role of antibody in immune recovery from infection with lymphocytic choriomeningitis virus (LCMV) strain WE was evaluated in B-cell-depleted mice. Mice were treated from birth with either affinity-purified rabbit anti-mouse immunoglobulin M (IgM), normal rabbit immunoglobulin, or, alternatively, an affinity-purified monoclonal rat anti-mouse IgM antibody (LO-MM-9); untreated mice served as controls. B-cell depletion was considered complete in specifically treated mice according to the following criteria: absence of a significant response to the B-cell mitogen lipopolysaccharide, absence of B cells expressing immunoglobulin on their surfaces, absence of detectable IgM or IgG in serum, and presence in the serum of free anti-IgM antibodies. In organs of μ-suppressed BALB/c mice, LCMV-WE replicated, dependent upon organ, at the same rate or more rapidly and, in general, to higher titers than in normal rabbit immunoglobulin-treated mice; untreated mice eliminated the virus most rapidly and showed lower virus titers. In addition, LCMV-primed control mice cleared a second LCMV challenge very rapidly and contained no virus by day 3, whereas μ-suppressed mice had virus in their blood and organs (except the spleen) up to days 3 to 6. The observed effects of anti-μ treatment may reflect the action of neutralizing antibodies (which so far have been difficult to demonstrate in vivo) or other antibody-dependent antiviral mechanisms which, together with T cells, efficiently control LCMV clearance.

The role of antibodies in recovery from or protection against virus infections varies with the virus and the route of infection (for reviews, see references 9, 19, 21, and 27). Overall, antibodies seem to play a major role in the defense against reinfection or the systemic spread of virus after reinfection. Their role in the control of virus replication, spread, and elimination during primary infection is much less clear.

During an infection with lymphocytic choriomeningitis virus (LCMV) strain WE, T cells have been demonstrated to efficiently control virus replication after days 5 to 6 (1, 4, 17, 28). The relative cytotoxic T-cell activity parallels virus titers, with a lag period of about 2 to 4 days. Usually, maximal virus titers are reached around days 6 to 10 and cytotoxic T cell maxima are observed around days 8 to 12, depending on the dose of virus and the mouse strain used (10, 17, 20). Antibodies can be detected in vitro by a complement-binding assay, an antibody-binding assay, or a neutralization assay (3, 11, 13, 15, 23). However, the role of antibodies in LCMV protection and clearance in vivo is unclear. LCMV infection in mice has been studied thoroughly with respect to the role of the T cell in mediating immunopathological disease (3, 10, 11, 15, 25). Several studies with μ-suppressed mice lacking antibodies and B cells have excluded a crucial role of antibodies in these various T-cell-dependent disease manifestations (including choriomeningitis, hepatitis, and footpad swelling) (6, 12, 26). In this study, we compared the capacities of μ-suppressed mice and mock-treated or untreated mice to control LCMV-WE replication and elimination during primary and secondary infection. Using two different protocols, we found that μ-suppressed mice had higher virus titers in blood and organs for a longer time, but eventually (after 3 to 4 weeks) μ-suppressed animals were also capable of eliminating LCMV-WE.

MATERIALS AND METHODS

Mice and their treatment. Inbred mice [C57BL/6 (*H-2*^{b}), C57BL/10 (*H-2*^{b}), BALB/c (*H-2*^{d}), B10.BR (*H-2*^{k})] and outbred NMRI mice were purchased from the Institut für Zuchthygiene, University of Zurich. Hybrid mice (C57 BL/6×B10.BR)F_1 were bred locally. Husbandry of mice and the induction and maintenance of B-cell suppression were performed exactly as described previously (5, 6). Briefly, anti-mouse immunoglobulin M (IgM) serum was produced in rabbits immunized with the mouse myeloma protein MOPC 104E (Bionetics, Kensington, England). Purification of anti-IgM (μ, lambda) (IRP) was achieved by using a column containing monoclonal mouse IgM (μ, lambda) coupled to Sepharose and eluted with 0.05 M glycine hydrochloride, pH 2.5. Protein content was measured according to the method of Lowry et al. (18), and reactivity against MOPC 104E was quantitated by solid-phase enzyme-linked immunosorbent assay. The generation, large-scale production, and purification of the monoclonal rat anti-mouse μ antibody LO-MM-9 has been described previously (2, 8). Affinity-purified LO-MM-9 and IRP were used at a concentration of 2 mg/ml. To initiate μ suppression, mice were treated with 0.1 ml of IRP or LO-MM-9 intraperitoneally on days 1, 2, 3, 5, 7, and 9 after birth. For maintenance of suppression, mice were injected with 600 μg of IRP or LO-MM-9 once every week. Offspring of anti-IgM-treated females were treated similarly; these offspring were not only B-cell depleted but also agammaglobulinemic (5) and were used in all experiments. Control mice were kept under identical conditions and were either injected with chromatographically purified rabbit IgG (NRP; Nordic, Tilburg, The Netherlands) or were left without treatment.

Monitoring of suppression. The assays used to demon-

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strate efficient B-cell depletion have been described in detail previously (5, 7). Briefly, spleen single-cell suspensions obtained from anti-IgM-treated and control animals were tested for the presence of surface immunoglobulin-positive cells by using a mixture of fluoresceinated goat anti-mouse μ and goat anti-mouse kappa conjugates (Tago Inc., Burlingame, Calif.) at appropriate dilutions. To assure that the labeled antibody was used at saturation, appropriate dilutions of commercially available fluoresceinated goat anti-mouse μ or kappa were determined by serial dilution of every batch. Under saturating conditions, about 40% of spleen cells from untreated adult C57/BL6 mice from our colonies were surface IgM positive. The percentage of positive cells was determined by counting the number of positive cells per 300 cells in a hemacytometer under a fluorescent microscope. The mitogen-induced proliferative response of single-cell suspensions of individual spleens was determined by culturing 5×10^5 cells in a volume of 200 μl in flat-bottomed microculture plates (Falcon 3072) in Iscove modified Dulbecco medium supplemented with antibiotics, glutamine, 2-mercaptoethanol, and 10% fetal calf serum. Triplicate cultures were set up either in the presence of lipopolysaccharide (LPS; 10 μl/ml; *Escherichia coli* O55:BS; Difco Laboratories, Detroit, Mich.) or concanavalin A (ConA; 2.5 μg/ml; Pharmacia, Uppsala, Sweden) or without mitogen. After 24 h, 2 μCi of [^{3}H]thymidine ([^{3}H]TdR) was added to each culture, and 6 h later, they were harvested with a Dynatech Automash cell harvester and counted in a liquid scintillation counter. Serum immunoglobulin determinations were performed by solid-phase enzyme-linked immunosorbent assay, using commercially available conjugates as described previously (7).

Lymphocytic choriomeningitis virus and titration of virus. The WE strain of LCM virus (22) was propagated and titrated either in L-cell cultures as PFU (16) or as 50% mouse infective doses (ID_{50} [14]). In a series of parallel titrations, 1 PFU has been found to correspond to about 10 ID_{50} (F. Lehmann-Grube, unpublished observation). Mice were infected by intravenous (i.v.) inoculation; at intervals they were bled from the retroorbital venous plexus. The blood was diluted 1:10 with Hanks balanced salt solution containing a trace of heparin and 1% heat-inactivated calf serum, sonicated for 30 s, poured into ampoules, and frozen at −70°C. Subsequently, the mice were killed by cervical dislocation and the organs were homogenized with known volumes of balanced salt solution containing 1% calf serum. The homogenates were cleared of debris by centrifugation, poured into ampoules or capped plastic tubes, and kept frozen at −70°C until titration. The methods used for titrating LCMV have been described in detail previously (17, 20).

RESULTS

Characterization of B-cell depletion. C57BL/6 mice treated with monoclonal rat anti-mouse IgM antibody LO-MM-9 had ≤2% surface immunoglobulin-positive spleen cells, compared with 35% in control mice (Table 1). Cultured spleen cells did not respond to the B-cell mitogen LPS and showed reduced [^{3}H[TdR] incorporation rates compared with untreated mice. Both anti-μ-treated and control spleen cells proliferated similarly in the presence of ConA. Anti-μ-treated mice had serum IgM levels below the detection limit of 3 μg/ml, and an excess of free rat anti-mouse μ antibody could be demonstrated in their sera.

Clearance of LCMV-WE from blood, organs, or footpads in IRP- and NRP-treated and control mice. Mice that had been μ suppressed by IRP treatment and NRP-treated or untreated control $(C57BL/6 \times B10.BR)F_1$ mice were infected i.v. with 3×10^4 ID_{50} of LCMV-WE. On days 5, 7, 9, 12, 16, and 20 thereafter, two or three mice were sacrificed; the blood was titrated by a plaque assay and organs were homogenized and freeze-thawed before the ID_{50} was assessed by a footpad assay (11). One experiment of two is shown in Fig. 1. Virus titers rose quicker and to higher titers and, most notably, persisted for longer times in IRP- than in NRP-treated mice. In turn, NRP-treated mice were slightly less efficient in controlling LCMV-WE replication than untreated controls.

When LCMV-WE elimination from footpads after local infection with a high dose of about 10^6 ID_{50} was tested, IRP-treated animals showed consistently but not significantly higher titers but only slightly differing kinetics of viral titers when compared with NRP-treated or untreated controls (Fig. 2). Late during infection on days 16 and 20, untreated mice had undetectable levels of virus, whereas both NRP- and IRP-treated mice still contained virus. However, virus was eliminated between 3 and 5 weeks after infection; several μ-suppressed mice monitored at that time did not contain LCMV in blood, spleen, or liver above the detection level of 2.7 $\log_{10}$ ID_{50} per g or per ml.

Clearance of LCMV-WE in blood of LO-MM-9-treated mice. Because of the apparent difference between untreated and NRP-treated mice, a different protocol for μ suppression, using monoclonal rat anti-mouse IgM, was applied as defined in Materials and Methods. Whereas IRP- or NRP-treated mice have been shown to possess activated macrophages, LO-MM-9 μ-suppressed mice did not when tested by their anti-*Listeria* bactericidal capacity (unpublished observation). When virus titers were determined in blood of LO-MM-9-treated and untreated BALB/c mice in two independent experiments with the footpad assay, a clear differ-

TABLE 1. Characterization of C57BL/6 mice treated with the monoclonal rat anti-mouse IgM antibody

Treatment[a]	Effect in spleen cells				Concn in serum[b] of:	
	% cell surface IgM-positive cells[c]	[^{3}H]TdR incorporation[d] with:			Mouse IgM	Free rat anti-μ
		No mitogen	LPS	ConA		
Anti-μ	≤2	12 ± 0.6	13.7 ± 2.3	157 ± 6.6	≤3	923 ± 230
None	34.7 ± 8	44.7 ± 2.1	171.1 ± 17	149 ± 12.5	306.7 ± 140	NT

[a] Groups of three C57BL/6 mice were injected with LO-MM-9 from birth as described in Materials and Methods or were left untreated. At the age of 4 to 6 weeks, mice were sacrificed to obtain individual spleens and sera.

[b] The concentrations of mouse IgM (in micrograms per milliliter) and free rat anti-mouse μ antibody (in micrograms per milliliter) in the sera of individual mice were determined by solid-phase enzyme-linked immunosorbent assay. Numbers are means ± standard deviations. NT, Not tested.

[c] Percent surface IgM-positive cells as determined by direct immunofluorescence done with single-cell suspensions (mean ± standard deviation).

[d] Single-cell suspensions of individual spleens were cultured in triplicate at a concentration of 5×10^5 cells/200 μl for 24 h. After the addition of 2 μCi of [^{3}H]TdR for 6 h, cultures were harvested. Numbers for mitogen represent the mean in counts per minute $\times 10^3$ (mean ± standard deviation). LPS was used at 10 μg/ml, and ConA was used at 2.5 μg/ml.

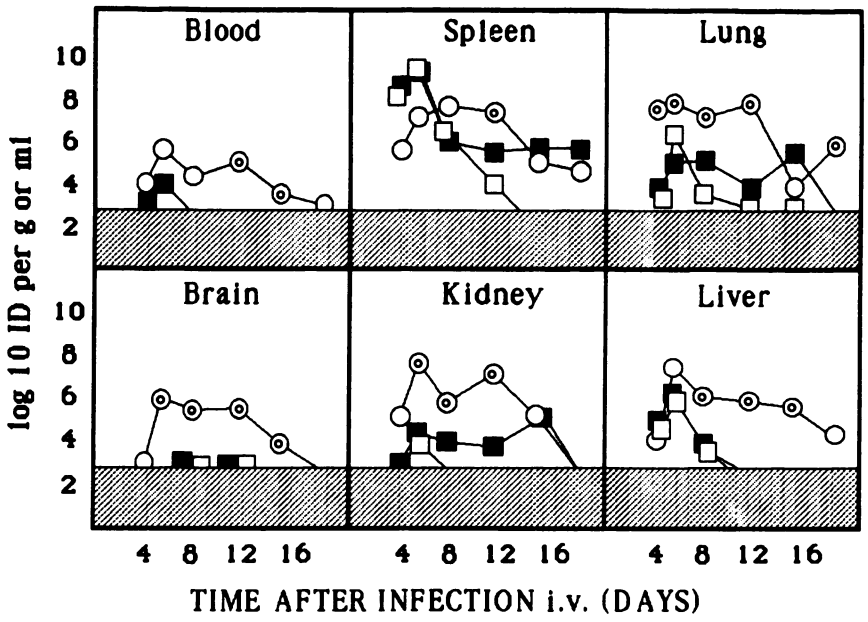

FIG. 1. Clearance of LCMV-WE from blood and organs after i.v. infection in IRP-treated and control mice. Groups of two or three IRP-treated (○), NRP-treated (■), or untreated (□) B10.BR or (C57BL/6×B10.BR)F_1 mice were injected with 3×10^4 ID_{50} of LCMV-WE and bled at the intervals indicated. Organ or blood samples obtained at different time points after infection were titrated with the footpad assay or by plaquing. The values given represent $\log_{10}$ of the mean ID_{50} per g of tissue or per ml of blood. The hatched area represents the detection threshold. Because of the small number of mice per group and time point, no statistical analysis is given; an inserted circle (◎) indicates where individual values of the IRP groups were nonoverlapping with the values of the other corresponding groups (NRP treated or untreated).

ence between LO-MM-9 μ-suppressed and control BALB/c mice was observed (Fig. 3). Again, generally higher titers persisting for longer times were observed in μ-suppressed mice.

Effect of LO-MM-9 μ suppression on LCMV replication during a second challenge infection with LCMV. BALB/c mice μ suppressed by treatment with LO-MM-9 or untreated control mice were immunized i.v. with LCMV-WE (2×10^2 ID_{50}) at the age of 5 weeks. Treatment with LO-MM-9 was continued and mice were challenged 12 weeks later with 2×10^5 ID of i.v. LCMV-WE. On days 3 and 6 after challenge,

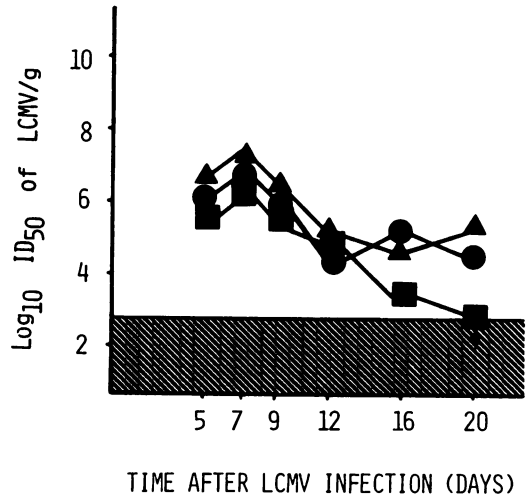

FIG. 2. Clearance of LCMV-WE from the footpad after local infection of IRP-treated and control mice. Groups of three IRP-treated (▲), NRP-treated (●), and untreated (■) B10.BR mice were injected in the footpad with 10^6 ID_{50} of LCMV-WE. The upper limit of the hatched area represents the detection threshold (2.7 $\log_{10}$ per g of tissue). The standard error of the mean of the values was <0.8 $\log_{10}$ for all values except day 12 untreated (1.7) and day 16 NRP treated (1.6). The only value significantly different ($P < 0.05$) was on day 20 untreated versus treated groups.

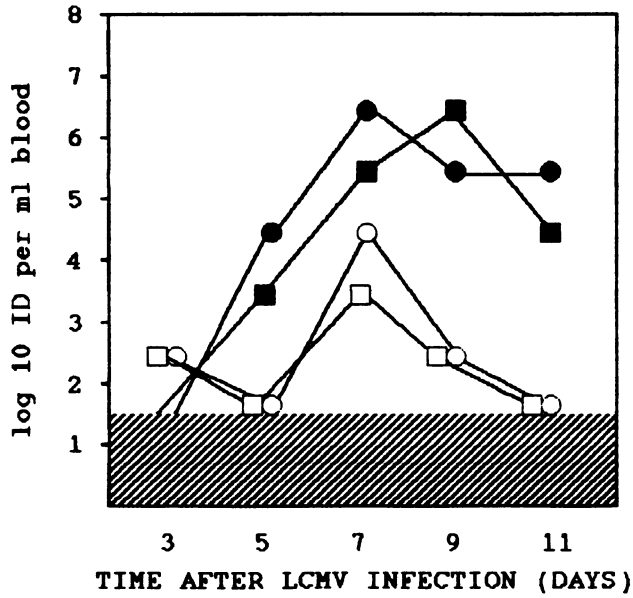

FIG. 3. Viremia after primary LCMV infection in LO-MM-9-treated and untreated mice. Groups of four anti-μ-treated (●, ■) or untreated (○, □) C57BL/6 mice were injected with 2×10^4 ID_{50} of LCMV-WE and bled at the intervals indicated. Blood samples of two mice were pooled (○, ●, □, ■) and the ID_{50} value was quantitated with the footpad assay. The detection threshold (hatched) was 1.5 $\log_{10}$ ID_{50} per ml of blood.

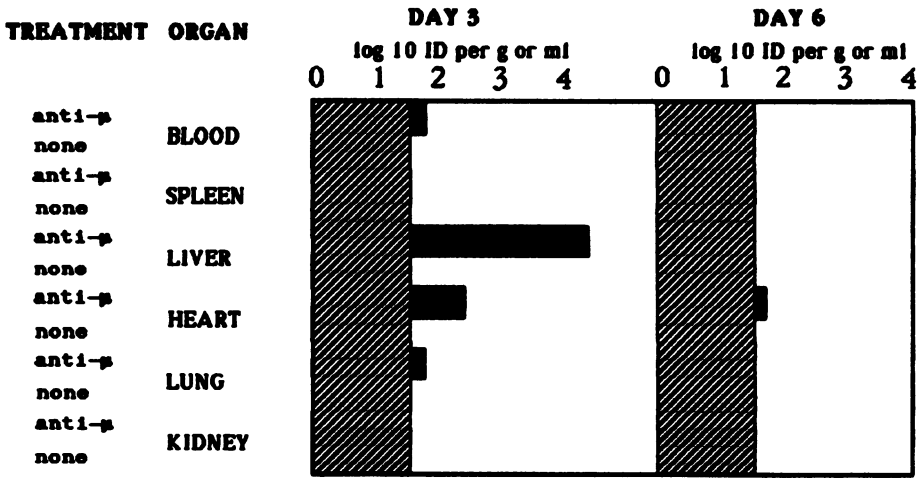

FIG. 4. Clearance of LCMV from blood and organs of primed LO-MM-9-treated and untreated mice. C57BL/6 mice either treated with monoclonal rat anti-mouse-μ antibody (anti-μ) or left without treatment (none) were injected with 3×10^2 ID_{50} of LCMV-WE and, 3 months later, challenged with 3×10^5 ID_{50} of the same virus. After 3 and 6 days, challenge groups of three mice were killed and organ homogenates were prepared and tested in the footpad assay. The values given represent $\log_{10}$ ID_{50} per g of the respective organ or per ml of blood. The detection threshold (hatched) was 1.5 $\log_{10}$ ID_{50}.

three mice per group were killed and LCMV titers were determined in blood and several organs (Fig. 4). LO-MM-9-treated, μ-suppressed mice had virus in blood, liver, heart, and lung on day 3 and in heart only on day 6, whereas none of the untreated LCMV-immune mice had detectable virus. These findings were confirmed in a second experiment (data not shown).

DISCUSSION

The experiments presented here demonstrate that antibody- and B-cell-deprived mice had some difficulties in controlling primary and secondary LCMV infections. LCMV reached higher titers and persisted for a longer time in μ-suppressed mice; however, the mice were able to eventually clear the virus successfully, but clearance was delayed compared with that for B-cell-competent mice. The results suggest some role of antibody in the control of LCMV growth, spreading, or both but indicate also that antibodies, although helpful, are not mandatory for complete LCMV elimination.

There is ample evidence in the literature that during LCMV infection antibodies are induced against various components of the virus (11, 15, 23–25). However, the role of antibodies in the control of LCMV infections is poorly understood.

In these studies, B-cell-deprived mice could not control LCMV titers in blood and organs as efficiently as B-cell-competent mice. These results may indicate that neutralizing antibodies that can be demonstrated in vitro (13, 23) function in vivo. Alternatively, these results suggest that other mechanisms involving B cells or antibodies may play a role, e.g., antibody-dependent cellular cytotoxicity or enhancement of phagocytosis and subsequent inactivation of virus.

The differences in initial replication kinetics of LCMV in μ-suppressed versus control treated or untreated mice are not understood. For example, titers in spleen and liver on days 4 and 5 (Fig. 1) were lower in μ-suppressed mice than in both control groups, whereas titers in lung, brain, kidney, and blood were higher in μ-suppressed mice. In another experiment (Fig. 3), viremia was initially lower in LO-MM-9-treated mice than in untreated controls. Effector T cells seem to be unimpaired in μ-suppressed mice (6, 7); macrophages were also found in function normally in LO-MM-9-treated mice but seemed to be activated in IRP- or NRP-treated mice when their functional state was evaluated by the capacity to clear and inactivate *Listeria monocytogenes* (A. Cerny, unpublished observation). Although these data seem to suggest that LCMV tropism and/or initial replication, as well as complete clearance, may be influenced by antibodies or other B-cell-dependent factors, this question remains unanswered. It must be kept in mind that complement (C')-dependent mechanisms have been found to inactivate LCMV in normal human serum but not in nonimmune mouse serum (24).

It is noteworthy that μ-suppressed mice rechallenged with LCMV had, in contrast to primed normal mice, considerable titers of virus on day 3 in various organs and also on day 6 in the heart. We tested LCMV-primed μ-suppressed mice earlier for their capacity to mount a footpad-swelling reaction upon rechallenge into the footpad, but we did not find a measurable reaction (6). This finding suggests that virus elimination from the footpad is rapid enough to limit virus replication and to restrict inflammatory reaction so as to be unmeasurable. The experiments presented here revealed a difference in the efficiency of primed mice to clear virus; μ-suppressed mice controlled challenge infections less well than control mice.

Overall, these experiments with μ-suppressed and with agammaglobulinemic mice demonstrate that T cells alone may eventually clear LCMV completely but that antibodies, although not mandatory, may accelerate virus clearance significantly.

ACKNOWLEDGMENTS

We thank F. Lehmann-Grube for the titration of LCMV shown in Fig. 1 and for generous support and encouragement.

This work was supported by grants from the Swiss National Science Foundation (3.323-0.82 and 3.259-0.85) and the Kanton Zürich and by grant no. 1316-C-187-B from the EURATOM. H.B. is a staff member of the Commission of the European Communities.

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