Immune Response against Lymphocytic Choriomeningitis Virus Infection in Mice without CD8 Expression

By Wai-Ping Fung-Leung,* Thomas M. Kündig,‡ Rolf M. Zinkernagel,‡ and Tak W. Mak*

*From the *Ontario Cancer Institute and Department of Medical Biophysics and Immunology, University of Toronto, Toronto, Ontario M4X 1K9, Canada; and the ‡Institute of Pathology, University of Zurich, Zurich, Switzerland*

Summary

The immune response against lymphocytic choriomeningitis virus (LCMV) was studied in a mutant mouse strain that does not possess $CD8^+$ T lymphocytes. Virus-specific cytotoxic T cell activity was generated in spleens of wild-type mice in an acute LCMV infection but was not measurable in mutant mice. Injection of replicating LCMV into footpads of wild-type mice induced a $CD8^+$ T cell–mediated swelling that peaked on day 8, followed by a $CD4^+$ T cell–mediated swelling that peaked on day 11, whereas mutant mice exhibited only the $CD4^+$ T cell–mediated swelling. After intracerebral inoculation with LCMV-Armstrong, all wild-type mice died of classical $CD8^+$ T cell–dependent choriomeningitis in 8–10 days. Mutant mice showed symptoms of general malaise but most of them survived. Mutant mice depleted of $CD4^+$ T cells by monoclonal antibody treatment showed no clinical signs of sickness. On day 9 after intravenous infection with LCMV-WE, virus was detected at high titers in spleens and livers of mutant mice but not in those of wild-type mice. On day 70 after injection of LCMV-WE into footpads, virus was not detected in wild-type mice and in one of the three mutant mice tested, but was still measurable in kidneys of the other two mutant mice. These results confirm in a new animal model that $CD8^+$ T cell–mediated immunity is crucial in LCMV clearance and in the immunopathological disease during LCMV infection. In addition, our results demonstrated a less severe form of choriomeningitis mediated by $CD4^+$ T cells and slow clearance of LCMV by alternative pathways independent of $CD8^+$ T cells.

$CD8^+$ T cells play an essential role in the immune response in mice against lymphocytic choriomeningitis virus (LCMV),[1] whereas antibodies of $CD4^+$ T cells do not seem to be crucially involved (1–4). A virus-specific cytotoxic activity mediated by $CD8^+$ T cells is generated in mice 7–9 d after an acute LCMV infection (5). $CD8^+$ T cells cause a swelling reaction 6–8 d after injection of replicating LCMV into footpads, whereas $CD4^+$ T cells are involved in the later phase of the swelling (6, 7). Intracerebral injection of LCMV into immunocompetent mice causes a fatal choriomeningitis that has been demonstrated to be mediated by CTL (8, 9). The respective roles of $CD8^+$ and $CD4^+$ T cells against LCMV infection have so far been studied in adoptive transfer experiments (2–4) and in vivo depletion experiments with mAbs against CD8 and CD4 (10–12). We have now generated a mutant mouse without CD8 expression by disrupting the Lyt-2 gene, which encodes one subunit of the CD8 heterodimer, with the technology of homologous recombination in embryonic stem cells (13). In this mouse strain, it has not been possible to demonstrate the presence of class I MHC-restricted cytotoxic T cells. For the study of the consequences of the anti-LCMV immune response without $CD8^+$ T cells, this new mouse model has several advantages compared to in vivo depletion experiments with mAbs. The mutant mice are devoid of $CD8^+$ T cells, and possible problems arising from antibody treatment such as incomplete depletion or stimulation instead of depletion of $CD8^+$ T cells can be excluded.

Evaluation of the immune response against LCMV in these CD8-defective mice confirms the key role of $CD8^+$ T cells in antiviral protection and immunopathology. Our results also reveal a $CD4^+$ T cell–dependent immunopathology after intracerebral infection with LCMV, and the clearance of LCMV through mechanisms independent of $CD8^+$ T cells.

[1] *Abbreviation used in this paper:* LCMV, lymphocytic choriomeningitis virus.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/91/12/1425/05 $2.00
Volume 174 December 1991 1425–1429

Materials and Methods

Mice and Viruses. The mutant mouse strain without CD8 expression was generated using the embryonic stem cell technology as described previously (13). These mice were H-2^b. C57BL/6 (H-2^b) mice were obtained from the breeding colony of the Institut fur Zuchthygiene (Tierspital Zurich, Switzerland). All mice were between 8 and 16 wk of age.

LCMV, strain WE (1) and strain Armstrong (2), were obtained from Dr. F. Lehmann-Grube (Hamburg, FRG) and Dr. M.B.A. Oldstone (La Jolla, CA), respectively. Production and titration of virus were performed on mouse L cells as described in detail previously (14). Titers of virus in infected organs were presented as PFU per gram of tissue.

Cytotoxicity Assay. 9 d after intravenous infection with LCMV-WE (200 PFU/mouse), spleen cells were assayed for LCMV-specific cytotoxicity in a standard ^{51}Cr release assay as described in detail elsewhere (15). The fibrosarcoma cell line MC57G (H-2^b) infected with LCMV-WE was used as target cells. YAC cells, which are sensitive to NK cell activity, and MC57G cells infected with vaccinia virus were used as control target cells for LCMV specificity. The assay was performed in duplicates with 4 h of incubation at 37°C, and specific lysis was calculated as described (9, 12).

Swelling Reaction of the Footpad and Assessment of Choriomeningitis. Swelling of footpads was measured daily with a spring-loaded caliper (1, 6, 7, 12) after 30 μl of LCMV-WE (500 PFU/footpad) were injected subcutaneously into the hind footpads. After intracerebral injection of 30 μl of LCMV-Armstrong (100 PFU/mouse), mice were monitored daily for lethality, clinical signs of choriomeningitis (2, 4, 8), and reduction of body weight. Change of body weight was calculated as: (body weight after infection − body weight before infection)/(body weight before infection).

In Vivo Depletion of $CD4^+$ or $CD8^+$ Cells. mAbs produced by hybridoma cell lines YTS 169.4 and YTS 191.1, which are specific for mouse CD8 (Lyt-2) and CD4 (L3T4), respectively, were obtained from Dr. H. Waldmann (Cambridge, UK), and were prepared as described (10). $CD4^+$ or $CD8^+$ T cells were depleted by intravenous injection with 1 mg of the respective antibody 3 and 1 d before 1 LCMV infection, as described (10).

Serotherapy with Polyclonal Anti-IFN-γ and anti-TNF-α. The production of sheep antisera against IFN-γ and TNF-α was described in detail elsewhere (16, 17). Daily, mice were injected intraperitoneally with 3 × 10^4 neutralizing units of anti-IFN-γ, anti-TNF-α, or normal sheep serum, from day 5 after intracerebral LCMV infection.

Results and Discussion

Antiviral Cytotoxic Activity during Acute LCMV Infection. Antiviral cytotoxicity activity of spleen cells was assayed for 9 d after intravenous injection of LCMV-WE into wild-type mice and mice homozygous and heterozygous for the disrupted Lyt-2 gene. Wild-type mice and heterozygous mutant mice showed similar cytotoxic activities towards LCMV-WE-infected MC57G cells, whereas nonspecific lysis of YAC cells and vaccinia virus–infected MC57G cells was minimal (Fig. 1). Specific cytotoxicity was not detected in homozygous mutant mice, but elevated levels of nonspecific lysis of YAC cells and vaccinia virus–infected MC57G cells were observed.

Primary Local Swelling Reaction after Intrafootpad Infection with LCMV. Subcutaneous inoculation of LCMV-WE into footpads of mice has been demonstrated to induce a virus-specific swelling reaction (6, 7, 18–20). Wild-type mice and heterozygous mutant mice exhibited an initial major swelling that peaked on day 8, followed by a later minor swelling phase that started at around day 10, and then slowly declined in inflammation (Fig. 2). The initial swelling has been shown to be mediated by $CD8^+$ T cells, whereas the later swelling was mediated by $CD4^+$ T cells (7, 19, 20). The $CD4^+$ T cell–mediated swelling shown in mutant mice confirms that

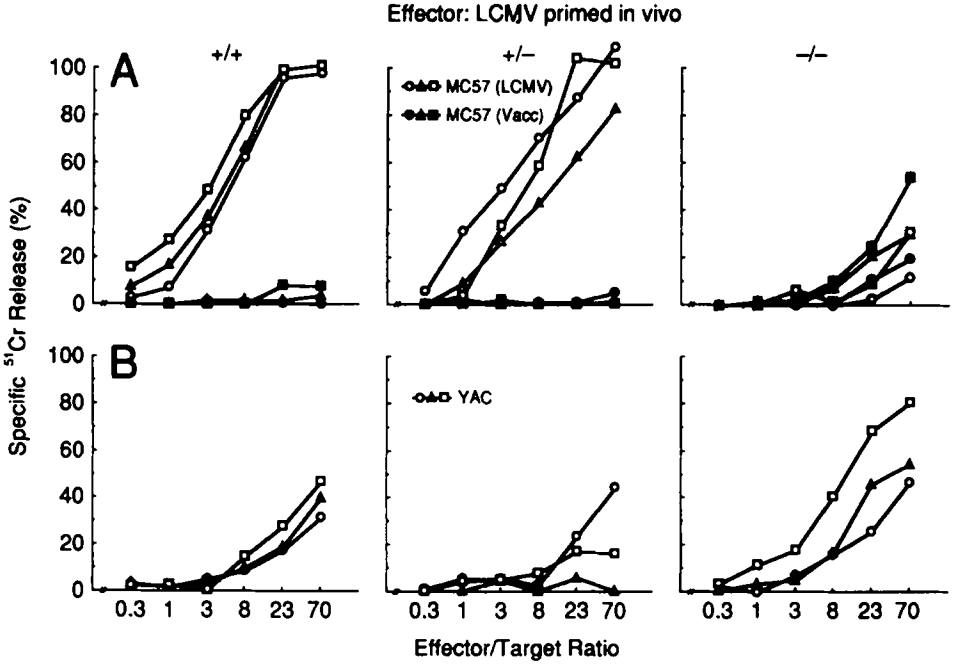

Figure 1. Anti-LCMV cytotoxic T cell activities in an acute LCMV-WE infection. Wild-type mice (+/+) and mice heterozygous (+/−) and homozygous (−/−) for the disrupted Lyt-2 gene were intravenously injected with LCMV-WE (500 PFU/mouse). Effector cells were from spleens of mice (H-2^b) on day 9 after infection are as described in Materials and Methods. Target cells were (*A*) MC57G cells either infected with LCMV-WE or vaccinia virus, and (*B*) YAC cells as control for NK cell activity.

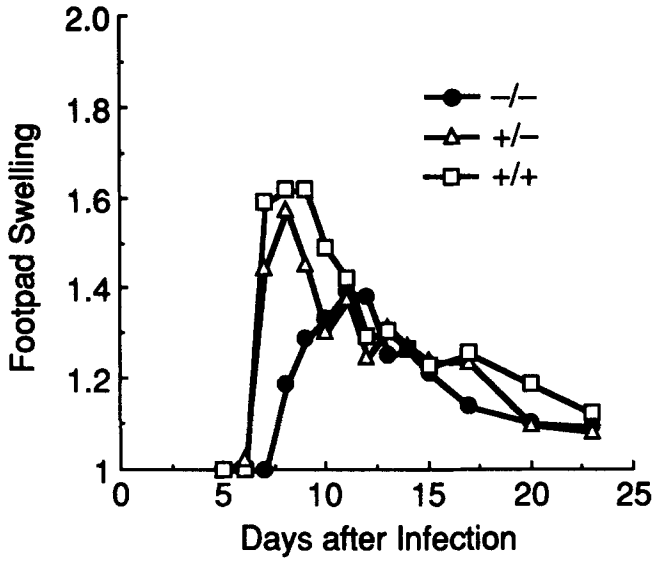

Figure 2. Footpad swelling reaction in mice after local infection with LCMV-WE. Swelling of footpads is expressed as folds of footpad thickness compared to that before inoculation. Data points represent means of six individual measurements from both hind feet of three mice.

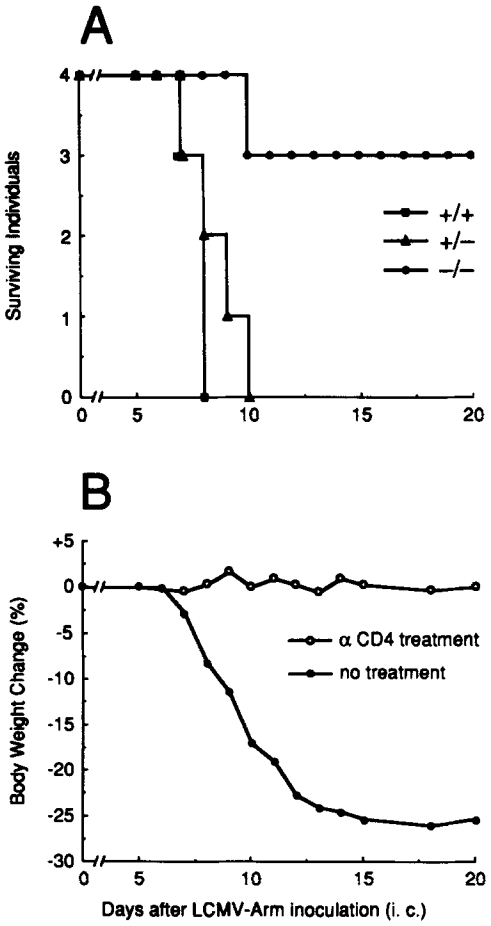

Figure 3. Choriomeningitis induced by intracerebral injection of LCMV-Armstrong (100 PFU/mouse) into mice. (*A*) Survival of wild-type mice (+/+) and mice heterozygous (+/−) and homozygous (−/−) for the disrupted Lyt-2 gene on different days after infection is shown. Groups of four mice were used. (*B*) Effect of anti-CD4 antibody treatment on body weight reduction in homozygous mutant mice. Anti-CD4 antibody (1 mg/mouse) was injected intravenously as described in Materials and Methods. Data points represent means of body weight change of two mice used in each group.

$CD4^+$ T cells can mediate local inflammation independent of $CD8^+$ T cells after subcutaneous injection with LCMV.

Development of Choriomeningitis after Intracerebral Infection with LCMV. Intracerebral inoculation with LCMV usually leads to fatal choriomeningitis that is not the result of a direct cytoplasmic effect of the virus but is caused by antiviral $CD8^+$ T cells (8, 9). After intracerebral infection of mice with LCMV Armstrong (100 PFU/mouse), all wild-type mice and heterozygous mutant mice showed severe neurological impairment starting on day 6 after inoculation and had died with characteristic convulsions by day 10 (Fig. 3 *A*). One out of four homozygous mutant mice died on day 10, the others survived but showed symptoms of general malaise and reduction of body weight. When homozygous mutant mice were treated with mAb to deplete $CD4^+$ T cells before intracerebral LCMV infection, they showed no signs of clinical sickness or body weight reduction, whereas mutant mice without antibody treatment were sick and lost 22% of their body weight, but they all survived (Fig. 3 *B*). Similar to the results shown in mutant mice, C57BL/6 mice (four mice tested) depleted of $CD8^+$ T cells by treatment with mAbs before intracerebral LCMV infection were temporarily sick but survived, whereas no symptoms were observed in C57BL/6 mice depleted of both $CD8^+$ and $CD4^+$ T cells (data not shown). Thus, our results demonstrate that in contrast to the fatal choriomeningitis mediated by $CD8^+$ T cells, $CD4^+$ T cells also mediate a less severe form of choriomeningitis in response to LCMV intracerebral infection. The time course and the severity of choriomeningitis as assessed by body weight reduction are comparable to the kinetics and the extent of the local footpad swelling reaction. This suggests that an acute and severe inflammation of the meninges mediated by $CD8^+$ T cells causes the death of mice, whereas the moderate and slow development of meningitis mediated by $CD4^+$ T cells leads to general malaise with significant body weight reduction but is not fatal. The $CD4^+$ T cell–mediated choriomeningitis indicated as body weight reduction was not eliminated by daily administration of polyclonal sheep anti-TNF-α nor anti-IFN-γ antiserum (data not shown). This treatment has been shown to be effective in abrogating $CD4^+$ T cell–mediated clearance of vaccinia virus in meninges (21) and clearance of Listeria in nude mice (22). This suggests that the $CD4^+$ T cell–dependent choriomeningitis in LCMV infection is mediated through other mechanisms.

Clearance of LCMV in CD8-defective Mice. LCMV is measurable in spleens of mice at high titers on day 4–6 after an acute infection, but most of the virus is eliminated by day 7–8, around the time point of maximal cytotoxic T cell response against viral antigen (23, 24). $CD8^+$ T cells have been shown to be the effector cells of cytotoxicity and are

crucial for the elimination of LCMV (3, 5, 11, 12, 25–28). On day 9 after intravenous infection with LCMV-WE, virus was detected at high titers in spleens and livers of the mutant mice, whereas wild-type mice and the heterozygous mutant mice had already cleared the virus to a level below detection (Table 1). For mice inoculated with LCMV-WE in footpads, virus was detected in blood samples of all three mutant mice but not in those of wild-type and heterozygous mutant mice on day 18 after infection. On day 70 after infection, all mice were killed and the organs, including spleen, liver, thymus, kidney, pancreas, ovary or testis, lung, and heart, were assayed for virus. Two of the mutant mice still had detectable virus in kidneys, but not in the other organs. One mutant mouse and all the wild-type mice had cleared the virus below detection levels (Table 1). Mutant mice that survived after intracerebral infection with LCMV-Armstrong were positive for virus in blood samples on day 18 after infection. However, on day 70 after infection, they were all negative in virus assays in all the organs mentioned above (data not shown). Taken together, these results confirm that $CD8^+$ T cells are crucial for clearance of LCMV after an acute infection. However, other cells may also be involved in LCMV clearance, although they eliminate LCMV at a considerably slower rate.

Table 1. *Titers of LCMV in Infected Mice*

Mouse strain (CD8 genotype)	LCMV-WE intravenous infection		LCMV-WE intrafootpad infection		
	Day 9 spleen	Day 9 liver	Day 18 blood*	Day 70 blood	Day 70 kidney
	PFU/g				*PFU/g*
−/−	2.8×10^7	4.2×10^6	+	+	4.0×10^4
	1.4×10^7	3.3×10^5	+	+	1.5×10^5
	2.3×10^7	1.7×10^5	+	−	$<10^2$
+/−	$<10^3$	$<10^2$	−	ND‡	$<10^2$
	$<10^3$	$<10^2$	−	ND	$<10^2$
	$<10^3$	$<10^2$	−	ND	$<10^2$
+/+	$<10^3$	$<10^2$	−	ND	$<10^2$
	$<10^3$	$<10^2$	−	ND	$<10^2$
	$<10^3$	$<10^2$	−	ND	$<10^2$

* Blood sample was regarded as "+", positive for the presence of virus, if a swelling reaction developed after injection of 30 μl of heparinized blood into the footpad of unprimed ICR ($H\text{-}2^q$) mice.
‡ Virus titer not determined.

We thank Irene Ng for preparing the manuscript, Maja Vollenweider and Dawn Grey for their technical help in breeding and typing of the CD8-defective mice, and Marco W. Schilham for critical reading.

Wai-Ping Fung-Leung is a research fellow of the Medical Research Council of Canada. This work was supported by grants from the Medical Research Council of Canada, National Science and Engineering and Research Council of Canada, and the National Cancer Institute of Canada.

Address correspondence to Dr. Tak W. Mak, Ontario Cancer Institute and Department of Medical Biophysics and Immunology, University of Toronto, 500 Sherbourne Street, Toronto, Ontario M4X 1K9, Canada. W.-P. Fung-Leung's present address is R. W. Johnson Pharmaceutical Research Institute, Don Mills, Ontario M3C 1L9, Canada.

Received for publication 26 June 1991.

References

1. Lehmann-Grube, F. 1971. Lymphocytic choriomeningitis virus. *Virol. Monogr.* 10:1.
2. Buchmeier, M.J., R.M. Welsh, F.J. Dutko, and M.B.A. Oldstone. 1980. The virology and immunobiology of lymphocytic choriomeningitis virus infection. *Adv. Immunol.* 30:275.
3. Lehmann-Grube, F., D. Moskophidis, and J. Löhler. 1988. Recovery from acute virus infection. Role of cytotoxic T lymphocytes in the elimination of lymphocytic choriomeningitis virus from spleens of mice. *Ann. NY Acad. Sci.* 532:238.
4. Doherty, P.C., and R.M. Zinkernagel. 1974. T cell-mediated immunopathology in viral infections. *Transplant. Rev.* 19:89.
5. Zinkernagel, R.M., and R.M. Welsh. 1986. H-2 compatibility requirement for virus-specific T cell-mediated effector functions in vivo. I. Specificity of T cells conferring antiviral protection against lymphocytic choriomeningitis virus is associated with H-2K and H-2D. *J. Immunol.* 117:1495.
6. Hotchin, J. 1962. The foot pad reaction of mice to lymphocytic choriomeningitis virus. *Virology.* 17:214.
7. Moskophidis, D., and F. Lehmann-Grube. 1989. Virus-induced delayed-type hypersensitivity reaction is sequentially mediated by $CD8^+$ and $CD4^+$ T lymphocytes. *Proc. Natl. Acad. Sci. USA.* 86:3291.
8. Cole, G.A., N. Nathanson, and R.A. Prendergast. 1972. Requirement for θ-bearing cells in lymphocytic choriomeningitis virus-induced central nervous system disease. *Nature (Lond.).* 238:335.
9. Baenziger, J., H. Hengartner, R.M. Zinkernagel, and G.A. Cole. 1986. Induction of prevention of immunopathological disease by cloned cytotoxic T cell lines specific for lymphocytic choriomeningitis virus. *Eur. J. Immunol.* 16:387.
10. Cobbold, S.P., A. Jayasuria, A. Nash, T.D. Prospero, and H. Waldmann. 1984. Therapy with monoclonal antibodies by elimination of T cell subsets in vivo. *Nature (Lond.).* 312:548.
11. Mokosphidis, D., S.P. Cobbold, H. Waldmann, and F. Lehmann-Grube. 1987. Mechanism of recovery from acute virus infections: treatment of lymphocytic choriomeningitis virus-infected mice with monoclonal antibodies reveals that $Lyt\text{-}2^+$ T lymphocytes mediate clearance of virus and regulate the antiviral antibody response. *J. Virol.* 61:1867.
12. Leist, T.P., S.P. Cobbold, H. Waldmann, M. Aguet, and R.M. Zinkernagel. 1987. Functional analysis of T lymphocytes subsets in antiviral host defense. *J. Immunol.* 138:2278.
13. Fung-Leung, W.-P., M.W. Schilham, A. Rahemtulla, T.M. Kündig, M. Vollenweider, J. Potter, W. van Ewijk, and T.W. Mak. 1991. CD8 is needed for development of cytotoxic T cells but not helper T cells. *Cell.* 65:443.
14. Lehmann-Grube, F., and J. Ambrassat. 1977. A new method to detect lymphocytic choriomeningitis virus-specific antibody in human sera. *J. Gen. Virol.* 37:85.
15. Zinkernagel, R.M., T. Leist, H. Hengartner, and A. Althage. 1985. Susceptibility to lymphocytic choriomeningitis virus isolates correlates directly with early and high cytotoxic T cell activity, as well as with footpad swelling reaction, and all three are regulated by H-2D. *J. Exp. Med.* 162:2125.
16. Leist, T.P., M. Eppler, and R.M. Zinkernagel. 1989. Enhanced virus replication and inhibition of lymphocytic choriomeningitis virus disease in anti-gamma interferon treated mice. *J. Virol.* 63:2813.
17. Leist, T.P., and R.M. Zinkernagel. 1990. Treatment with anti-tumor-necrosis factor-alpha does not influence the immune pathological response against LCMV. *Cytokines.* 2:29.
18. Zinkernagel, R.M. 1976. H-2 restriction of virus-specific T cell mediated effector functions in vivo II. Adoptive transfer of delayed type hypersensitivity to murine lymphocytic choriomeningitis virus is restricted by the K and D region of H-2. *J. Exp. Med.* 144:776.
19. Moskophidis, D., L. Fang, J. Gossmann, and F. Lehmann-Grube. 1989. Mechanism of recovery from acute virus infection. IX clearance of lymphocytic choriomeningitis (LCM) virus from the feet of mice undergoing LCM virus-specific delayed-type hypersensitivity reaction. *J. Gen. Virol.* 70:3305.
20. Moskophidis, D., L. Fang, J. Gossmann, R. Drjubin, J. Löhler, M. Bruns, and F. Lehmann-Grube. 1990. Virus-specific delayed-type hypersensitivity (DTH). Cell mediating lymphocytic choriomeningitis virus-specific DTH reaction in mice. *J. Immunol.* 144:1926.
21. Binder, D., and T.M. Kündig. 1991. Antiviral protection by $CD8^+$ versus $CD4^+$ T cells, $CD8^+$ T cells correlating with cytotoxic activity in vitro are more efficient in antivaccinia protection than CD4-dependent interleukins. *J. Immunol.* In press.
22. Hauser, T., K. Frei, R.M. Zinkernagel, and T.P. Leist. 1990. Role of tumor necrosis factor in Listeria resistance of nude mice. *Med. Microbiol. Immunol.* 179:95.
23. Marker, O., and M. Volkert. 1973. Studies on cell-mediated immunity to lymphocytic choriomeningitis virus in mice. *J. Exp. Med.* 137:1511.
24. Lehmann-Grube, F., V. Assmann, C. Löliger, D. Moskophidis, and J. Löhler. 1985. Mechanism of recovery from acute virus infection in role of T lymphocytes in clearance of lymphocytic choriomeningitis virus from spleens of mice. *J. Immunol.* 134:608.
25. Assmann-Wischer, U., M.M. Simon, and F. Lehmann-Grube. 1985. Mechanism of recovery from acute virus infection. III. Subclass of T lymphocytes mediating clearance of lymphocytic choriomeningitis virus from the spleens of mice. *Med. Microbiol. Immunol.* 174:249.
26. Moskophidis, D., U. Assmann-Wischer, M.M. Simon, and F. Lehmann-Grube. 1987. The immune response of the mouse to lymphocytic choriomeningitis virus V. High numbers of cytolytic T lymphocytes are generated in the spleen during acute infection. *Eur. J. Immunol.* 17:937.
27. Klavinskis, L.S., R. Geckeler, and M.B.A. Oldstone. 1989. Cytotoxic T lymphocyte control of acute lymphocytic choriomeningitis virus infection: interferon gamma, but not tumour necrosis factor alpha, displays antiviral activity in vivo. *J. Gen. Virol.* 70:3317.
28. Byrne, J.A., and M.B.A. Oldstone. 1984. Biology of cloned cytotoxic T lymphocytes specific for lymphocytic choriomeningitis virus: clearance of virus in vivo. *J. Virol.* 51:682.