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Antiviral Immune Responses of Lymphocytic Choriomeningitis Virus-Infected Mice Lacking CD8$^+$ T Lymphocytes because of Disruption of the β_2-Microglobulin Gene

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Mice infected intracerebrally with lymphocytic choriomeningitis virus (LCM virus) develop a characteristic central nervous system disease and usually die. If the intravenous or intraperitoneal route is used, the infection leads to less severe clinical signs and the virus is eliminated. Illness and virus clearance are immunological phenomena, which are assumed to be caused exclusively by CD8$^+$ T lymphocytes. In contrast, of the two phases of a delayed-type hypersensitivity reaction caused by inoculation of the virus into the mouse's foot, only the first is mediated by CD8$^+$ cells, whereas the second is mediated by CD4$^+$ cells. We have examined LCM virus-specific immune responses in mice devoid of CD8$^+$ T lymphocytes as a result of disruption of the β_2-microglobulin gene. As expected, the virus persisted but footpad swelling did not occur, although intracerebral infection resulted in CD4$^+$ T-lymphocyte-mediated illness and antiviral antibodies were produced. Different results had been obtained by Fung-Leung et al. (W.-P. Fung-Leung, T. M. Kündig, R. M. Zinkernagel, and T. W. Mak, J. Exp. Med. 174:1425–1429, 1991), who, in essentially identical experiments but with mice lacking CD8$^+$ T lymphocytes as a result of disruption of the Lyt-2-encoding gene, recorded control of the infection and development of a local delayed-type hypersensitivity reaction. We consider these differences important, because they provide us with clues that may help to understand the mode of action of the CD8$^+$ T cells in cell-mediated antiviral immunity.

Disease signs following infection of adult mice with lymphocytic choriomeningitis virus (LCM virus) virus are pathological immune phenomena, in which CD8$^+$ T lymphocytes that are restricted by class I molecules encoded by the major histocompatibility complex (MHC) play prominent roles (reviewed in references 7, 12, and 27). The same type of cell is also assumed to mediate elimination of the virus from the tissues of surviving animals (reviewed in references 33 and 62). Although these general rules are probably accepted by most people working in the field of viral immunology, there is less agreement about the underlying mechanisms. When mutant mice devoid of CD8$^+$ T lymphocytes became available, it was hoped that the analysis of their response to LCM virus would help answer some of the still open questions. Here, we present our findings obtained with mice whose lack of CD8$^+$ T cells results from their inability to synthesize β_2-microglobulin (β_2m) and to form class I molecules on cell surfaces (60). Similar experiments with the same virus but with mice free of CD8$^+$ cells as a result of disruption of the *Lyt-2* gene had been reported by Fung-Leung et al. (17). Although there are similarities in the results, there are also differences, two of which are especially noteworthy. Whereas the mice used by us did not terminate the infection and did not develop delayed-type hypersensitivity (DTH) as revealed by local inflammation after inoculation of the virus into the foot, the Lyt-2-lacking mice eliminated the virus and exhibited a DTH footpad reaction.

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MATERIALS AND METHODS

Mice. *H-2^b* mice that are homozygous for β_2m-disrupted genes (−/−) and their +/+ counterparts (61) were given to us by Rudolf Jaenisch, Whitehead Institute for Biomedical Research, Boston, Mass. They were specific pathogen free on receipt and were maintained and bred by us under strict barrier conditions. For the experiments, male and female mice aged 8 to 16 weeks were used.

Virus. The WE strain LCM virus used here (51) is identical with the one used by Fung-Leung et al. (17). Presumably, the same is true with respect to strain Armstrong, although its origin is uncertain (26). Both viruses were propagated in L (NCTC clone 929) cells and quantitated as PFU in L cells (29). Because mice are more susceptible than L-cell cultures and are less sensitive to nonviral constituents, the mouse assay was used for the detection of low levels of infectious virus in tissue homogenates. According to the results of comparative titrations in mice and L-cell cultures, the 50% infectious doses of WE and Armstrong strain LCM viruses determined in mice were, respectively, 10 and 40 times higher than the numbers of PFU determined in cells (25a). On this basis, PFU were converted to mouse infectious units (MIU) by multiplication by 10 and by 40. For determining infectious titers in tissues, weighed portions were homogenized with known volumes of balanced salt solution by using a mortar and pestle and some sand.

L. monocytogenes. *Listeria monocytogenes* EGD (39) was supplied by H. Hahn, Institut für Medizinische Mikrobiologie, Freie Universität, Berlin, Germany. The bacteria were propagated in suspension and quantitated as CFU in tryptic soy broth and on tryptic soy agar (Difco Laboratories, Detroit, Mich.).

Evaluation of lymphocytic choriomeningitis. Beginning on day 5 after intracerebral (i.c.) or intraperitoneal (i.p.) inoc-

ulation of 10^5 MIU of LCM virus Armstrong and WE, respectively, clinical signs and histological alterations were recorded at intervals.

Cytotoxicity assay. The chromium release assay of Brunner et al. (6) was modified by using microcups and an incubation time of 4 h at 37°C. Target cells for LCM virus-specific $CD8^+$ and $CD4^+$ cytotoxic T lymphocytes (CTL) were infected simian virus 40-transformed C57BL/6J fibroblastic cells (established by us) and CH2B B-cell lymphoma cells (46), respectively.

Footpad reaction. Beginning 4 days after the subcutaneous inoculation of 10^5 MIU of WE strain virus into the right hind footpad, the dorsoventral thicknesses of both feet were measured with calipers at regular intervals. Swelling is expressed as the factor by which the thickness of the inoculated foot exceeded the thickness of the contralateral foot (28). For determining listeria-specific DTH, mice were infected with 5×10^3 CFU and challenged 7 days later by footpad inoculation of listerial antigen (21), which had been given to us by S. H. E. Kaufmann, Institut für Mikrobiologie der Universität Ulm, Ulm, Germany. Footpad thicknesses were measured and expressed as for LCM virus.

Quantitation of antiviral antibodies. The solid-phase immunoenzymatic technique for enumerating single cells forming antiviral antibodies and the enzyme-linked immunosorbent assay (ELISA) to measure antibodies in the circulation have been described previously (45). Neutralizing titers were determined by a plaque reduction test (23).

MAbs. All monoclonal antibodies (MAbs) used were of rat origin. For identification by fluorescence-activated cell sorting of $CD4^+$ and $CD8^+$ cells, MAbs GK1.5 (10) and 53-6.7 (25), respectively, were used. Immunostaining of CD8 in spleen sections was done with MAb M1/42.3.9.8 HLK (22). Depletion of $CD4^+$ T lymphocytes in vivo ("serologic surgery") was accomplished by injecting i.p. a mixture of CD4 MAbs YTS 191.1 and YTA3.1 (49). For control purposes, MAb YTS 169 was inoculated i.p., a procedure that effectively depletes mice of $CD8^+$ T lymphocytes (42).

Histological and immunocytochemical methods. Tissue specimens were fixed in 4% paraformaldehyde containing 1% acetic acid and embedded in Histosec (Merck, Darmstadt, Germany). Sections were stained with hematoxylin and eosin, periodic acid-Schiff stain, or Giemsa stain. When required, bones were decalcified with saturated EDTA solution before being embedded.

For the detection of CD8 on cells, pieces of spleen were snap-frozen at −70°C and cryosectioned. The sections were incubated for 60 min with suitably diluted MAb, washed with Tris-buffered saline, incubated with biotinylated $F(ab')_2$ fragments of immunoaffinity-purified mouse antibody against rat immunoglobulin G (Jackson Laboratory, Avondale, Pa.), immersed in a solution of streptavidin biotin-peroxidase complexes (Dakopatts, Copenhagen, Denmark), and subjected to the diaminobenzidine reaction.

RESULTS

Search for $CD8^+$ T lymphocytes in the β_2m-free mice. Analysis of lymphoid cells from uninfected β_2m −/− mice by fluorescence-activated cell sorting, as well as cytochemical inspection with MAbs, revealed $CD4^+$ cells but no $CD8^+$ cells in −/− mice. However, in the inflammation that developed during infection with LCM virus, a few $CD8^+$ T lymphocytes were present. Essentially the same was observed by Zijlstra et al., who did not detect these cells by flow-cytometric analysis in naive β_2m-lacking mice but found a few in mice that had rejected a skin transplant (59). In wild-type mice the usual patterns were observed (data not shown).

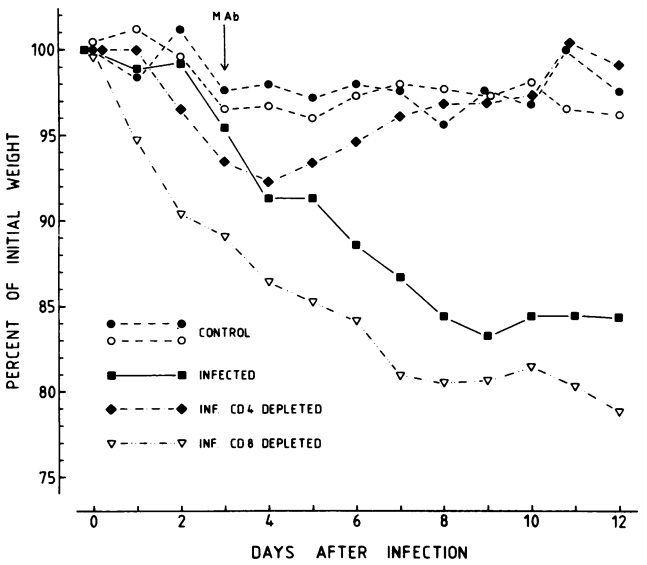

FIG. 1. Loss of weight of LCM virus-infected β_2m-deficient mice and its reversal by depleting them of $CD4^+$ T lymphocytes. Groups of mice were infected i.c. with 10^5 MIU of strain Armstrong LCM virus or were infected and treated 3 days later by i.p. inoculation of a mixture of CD4 MAbs YTS 191.1 and YTA3.1. Control mice were left uninfected and untreated or were infected and treated with MAb YTS169, which depletes ordinary mice of $CD8^+$ cells. Each datum point represents the mean of five measurements, and solid and open symbols signify different experiments.

Search for LCM virus-specific lytic activity in infected mice. In repeated attempts, no LCM virus-specific CTL activity restricted by MHC-encoded class I molecules could be detected in infected −/− mice, which confirms findings with other immunogens (14, 35, 60); a normal response was obtained in +/+ mice.

Prompted by a recent report by Muller et al. (46), who detected LCM virus-specific $CD4^+$ CTL in infected β_2m-lacking mice of another strain, we searched for a corresponding activity in our mice. Despite the use of similar target cells, we found none in a number of trials.

Lymphocytic choriomeningitis. To study the disease lymphocytic choriomeningitis, we inoculated mice i.c. with 10^5 MIU of Armstrong LCM virus and inspected them at 24-h intervals. (Virus of this strain was chosen because it causes a more severe disease than the WE LCM virus [26]. Furthermore, it does not induce "high-dose immune paralysis" [26], which might have complicated the findings.) Whereas the +/+ control animals suffered from a characteristic illness leading to death within 7.1 ± 0.3 days (mean ± standard error, $n = 17$), 20 similarly infected −/− mice showed signs of a minor illness for about 2 days; they lost 15 to 20% of their weight (Fig. 1; a further experiment led to essentially the same results), and the histological inspection revealed a moderate inflammation of the leptomeninges 8 days after infection (Fig. 2A); in choroid plexuses and ependyma there were only minimal infiltrates, and destructive lesions, which are typical for the neurological LCM virus disease, were absent. Two weeks later, the meningeal infiltrates had completely resolved, leaving behind a vacuolar degeneration of white matter in areas that had been in close contact with the

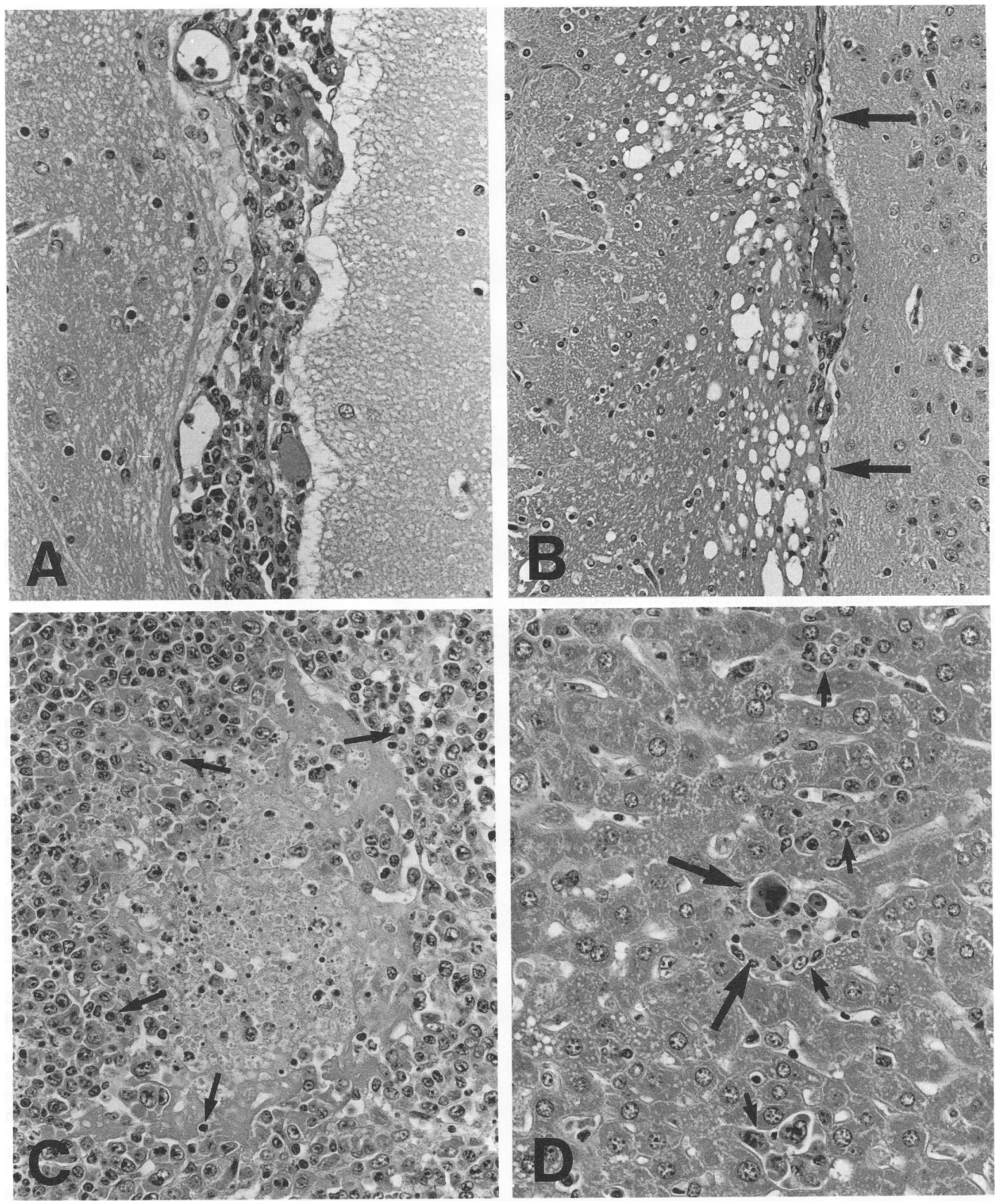

FIG. 2. Histopathological changes in β_2m-lacking mice after infection with LCM virus. All panels are stained with hematoxylin and eosin. (A) Mononuclear inflammatory infiltrate in the pia-arachnoid of the fissura chorioidea 8 days after i.c. inoculation of 10^5 MIU of strain Armstrong LCM virus. Magnification, ×360. (B) Vacuolar degeneration of the crus cerebri 30 days after i.c. inoculation of 10^5 MIU of strain Armstrong LCM virus; arrows point to the adjacent pia-arachnoid. Magnification, ×230. (C) Fibrinoid necrosis of splenic white pulp 8 days after i.p. inoculation of 10^5 MIU of strain WE LCM virus; arrows point to lymphoid cells undergoing apoptotic cell death. Magnification, ×360. (D) Mild form of hepatitis 8 days after i.p. inoculation of 10^5 MIU of strain WE LCM virus; large arrows point to eosinophilic single-cell necroses of hepatocytes, and small arrows point to intrasinusoidal mononuclear infiltrates. Magnification, ×360.

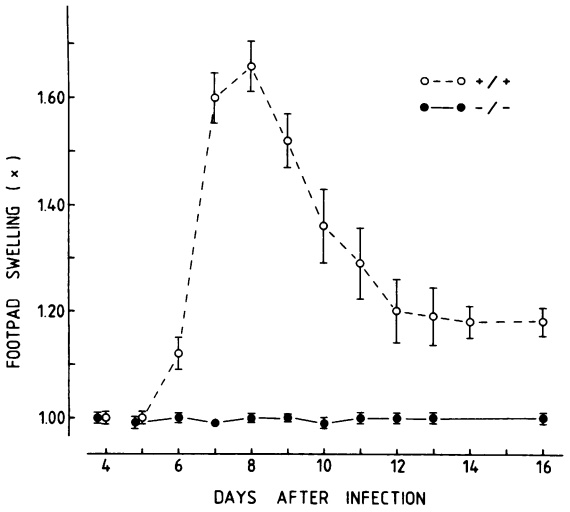

FIG. 3. Absence of LCM virus-specific DTH footpad swelling reaction in β_2m-lacking mice. Beginning 4 days after subcutaneous injection of 10^5 MIU of WE strain LCM virus into the right hind feet and at 24-h intervals thereafter, both hind feet were measured. Circles and bars denote, respectively, means and standard errors in seven mice of the factors by which the thicknesses of the right feet exceeded the thicknesses of the left feet.

pia-arachnoid (Fig. 2B). In i.c.-infected −/− mice, depletion of CD4+ T lymphocytes by injection of CD4-specific MAb prevented the development of clinical signs and histological alterations. The beneficial effect of the removal of CD4+ T lymphocytes was confirmed by weight measurements; treatment with a depleting CD8 MAb had no such result (Fig. 1). In +/+ mice, the pathological consequences of the infection were not measurably altered by depletion of CD4+ cells.

Mice were also infected i.p. with 10^5 MIU of WE strain LCM virus, which is more viscerotropic than the Armstrong strain (26). In −/− mice the histological examination disclosed fibrinoid necroses of the splenic white pulp (Fig. 2C) and a mild hepatitis (Fig. 2D), which were maximal on day 8 after virus inoculation. The alterations in +/+ control animals were of the same kind, although much more severe; these have been described previously (37).

DTH reaction. The local inflammatory reaction following inoculation of the LCM virus into the footpads of mice (20) has been shown to be mediated sequentially by CD8+ and CD4+ T lymphocytes (43). Since in −/− mice there is no lack of the latter, we had expected swelling of the inoculated foot, albeit diminished and retarded. In two otherwise identical experiments, one with groups of seven mice (Fig. 3) and the other with groups of six (not shown), no swelling at all was observed. Histological inspections of the tissues of six −/− mice (two animals on days 7, 8, and 9 after inoculation, respectively) disclosed a few scattered mononuclear cells of uncertain relevance. We conclude that the β_2m-free mice did not develop LCM virus-specific DTH as revealed by the footpad reaction.

We wanted to know whether this deficiency was a general phenomenon, and so we tested the β_2m −/− mice for listeria-specific DTH. Seven days after infection with *L. monocytogenes*, mice were footpad challenged with listerial antigen, and thicknesses of the feet were measured 24, 48, and 72 h thereafter. In five −/− mice the factors of swelling at 24, 48, and 72 h were (means ± standard errors) 1.64 ± 0.10, 1.30 ± 0.03, and 1.15 ± 0.01, respectively, whereas in five wild-type controls the corresponding values were 1.85 ± 0.16, 1.55 ± 0.09, and 1.24 ± 0.03. The differences between the two groups of mice were just significant ($P > 0.05$, $P < 0.05$, and $P < 0.05$ for the three measuring intervals). When, 15 days later, LCM virus was inoculated into the footpads of these mice, no swelling occurred in the −/− mice, whereas marked local responses were seen in their +/+ counterparts.

LCM virus-specific antibodies. Levels of circulating antibodies were measured by ELISA. As the data in Table 1 show, virus-specific immunoglobulin M and immunoglobulin G were detectable 7 days after intravenous (i.v.) infection with 10^3 MIU. Antibodies appeared somewhat more slowly in the −/− than in the +/+ mice, but similar titers were eventually attained. This difference is not necessarily a reflection of the numbers of cells that partake in antibody production, because early (and also late) in infection more were found in the spleens of infected −/− than +/+ mice (Table 2).

We also searched for neutralizing antibodies. On the basis of previous results (23), the mice were infected by subcutaneous inoculation of 10^5 MIU and challenged i.v. with 10^7 MIU 16 days later. According to the data in Table 3, neutralizing antibodies appeared slowly and the titers remained low, which seems to be a characteristic of murine LCM virus infection (23). Because of the small number of mice tested, the finding that the neutralizing activity appeared earlier and attained higher titers in −/− than in +/+ mice may not signify a true difference, but the −/− mice are certainly quite capable of generating LCM virus-neutralizing antibodies.

Control of infection. The central question was whether mice without CD8+ T lymphocytes would nonetheless control the virus. The experimental answer is negative, and infectious titers remained high up to 100 days after virus inoculation (Table 4). In fact, an equilibrium formed between naturally decaying and excreted virus on one hand and new

TABLE 1. LCM virus-specific antibodies in sera of β_2m-lacking mice and their +/+ counterparts determined by ELISA

Day after infection[a]	Titer of circulating antibody (no. of mice)[b]			
	IgM		IgG	
	−/− mice	+/+ mice	−/− mice	+/+ mice
0 (control)	<30 (4)	<30 (4)	<30 (4)	<30 (3)
7	400 ± 129 (4)	1,268 ± 467 (5)	1,600 ± 516 (4)	9,051 ± 1,875 (4)
14	200 ± 91 (3)	200 ± 91 (4)	9,514 ± 2,020 (4)	22,627 ± 4,686 (4)
21	119 ± 39 (4)	<30 (3)	32,000 ± 3,037 (4)	64,000 ± <1 (4)

[a] By i.v. inoculation of 10^3 MIU of WE strain LCM virus.
[b] Geometric mean ± standard error.

TABLE 2. Numbers of spleen cells forming antibodies against LCM virus during primary infection of β_2m-lacking mice and their +/+ counterparts

Day after infection[a]	Strain of mice	No. of antibody-forming cells[b]			
		IgM		IgG	
		Per 10^6 cells[c]	Per spleen[d]	Per 10^6 cells	Per spleen
0 (control)	−/−	<[e]	<	<	<
	+/+	<	<	<	<
7	−/−	2,600, 2,800	197,600, 162,000	5,450, 6,800	414,200, 394,400
	+/+	2,650, 2,900	166,950, 208,800	3,550, 3,800	241,400, 277,400
8	−/−	6,800, 5,550	564,400, 438,450	9,800, 10,100	813,400, 797,900
	+/+	4,450, 5,050	289,250, 313,100	6,200, 6,450	483,600, 522,450
9	−/−	5,550, 4,900	445,500, 416,500	10,400, 8,750	842,400, 743,750
	+/+	2,600, 3,800	188,800, 258,400	5,550, 5,00	366,300, 355,000

[a] By i.v. inoculation of 10^3 MIU of WE strain LCM virus.
[b] Determined in two mice.
[c] Based on numbers of trypan blue-excluding cells.
[d] Based on total numbers of cells counted with Türk's solution.
[e] Below detectability.

production on the other, which makes these mice similar to carrier mice (32). The +/+ mice had no difficulty in eliminating the virus. Some infectivity was detected in spleens and livers long after virus injection, but LCM virus often persists, even when it has been injected into immunologically mature mice (reviewed in reference 54).

Infectious virus was also quantitated in the feet 20 days after local inoculation of 10^5 MIU. Whereas in +/+ mice the titer was down to approximately 2×10^4 MIU/g (average of six mice), in six −/− mice $6.12 \pm 1.18 \times 10^7$ (mean ± standard error) MIU/g was measured. These values are very similar to the ones found in the feet of mice of other strains untreated and depleted of $CD8^+$ T lymphocytes, respectively (44). Finally, large quantities of infectious virus were detected in brains, spleens, livers, and kidneys of 10 individual β_2m-deficient mice that had been inoculated i.c. 110 days previously with 10^5 MIU of strain Armstrong LCM virus.

DISCUSSION

The knowledge that illness and death of LCM virus-infected mice as well as termination of the infection are mediated exclusively and that the footpad swelling reaction is mediated in part by $CD8^+$ T lymphocytes rests on broad experimental evidence (11, 13, 42–44, 63, 64). Thus, the prediction was that adult mice devoid of these cells would (i) remain free of illness after i.c. or peripheral infection, (ii) not eliminate the virus after injection by any route, and (iii) respond with a $CD4^+$ cell-mediated DTH reaction. It was also expected that antiviral antibodies would be made (1, 42). Indeed, the $CD8^+$ cell-free mice used by us proved incapable of controlling the infection and produced antibodies; however, they developed illness after i.c. and i.p. injection of the virus and failed to express a DTH reaction in the footpad. Contrasting findings had been reported by Fung-Leung et al., who, in similar experiments with other $CD8^+$ cell-free mice, observed elimination of the LCM virus and a local DTH reaction (17). Thus, the two strains of mice lacking $CD8^+$ T cells, albeit for dissimilar reasons, respond differently to the LCM virus.

Clinical signs following i.c. inoculation of the Armstrong LCM virus, despite the absence of CTL, were seen by both groups of investigators, and both agree that these are caused directly or indirectly by $CD4^+$ T lymphocytes. The same cells are also responsible for the hepatitis seen in the −/− mice after i.p. inoculation of strain WE LCM virus (36) and presumably also for the splenic lesions. In this connection, the recent report of Muller et al. is relevant (46). They searched for virus-specific CTL in LCM virus-infected β_2m −/− mice (a different strain from the one used here) and found none directed against virus plus MHC class I molecules. However, class II-restricted CTL appeared, and the authors blamed this for the pathology that developed during infection (46). It is reasonable to assume that the same type of cells is responsible for the pathological alterations in our mice as well as in the CD8-lacking mice of Fung-Leung et al. (17), although our search for $CD4^+$ CTL was in vain. The pathology described by Muller et al. (46) was much more severe than the pathology seen by us, and our inability to demonstrate such cells may simply mean that they remained below the threshold of detection. Quantitative differences of LCM virus cell-mediated immune responses between mice of the same haplotype but differing in the non-MHC background have been described repeatedly (28, 31, 53).

Besides $CD4^+$ T lymphocytes, other cells seem to contribute to the pathology in −/− mice. As shown in Fig. 1, loss of weight is apparent 2 days after i.c. infection; this is unlikely to be the consequence of a virus-specific immunopathological response. Alterations appearing as early in lymphoid organs and livers of LCM virus-infected ordinary mice are probably caused by activated natural killer cells (31, 36).

TABLE 3. LCM virus-neutralizing antibodies in sera of β_2m-lacking mice and their +/+ counterparts

Week after challenge[a]	Neutralizing titer in mouse strain[b]:	
	−/−	+/+
0 (control)	<20, <20	<20, <20
3	40, 40	<20, 40
6	40, 40	<20, <20
9	80, 40	<20, <20
12	160, 160	40, 40
15	320, 80	80, 80

[a] Mice were infected by subcutaneous inoculation of 10^5 MIU and challenged i.v. 16 days later with 10^7 MIU.
[b] Neutralizing titer in sera from two mice per group. The titer is defined as the reciprocal of the serum dilution that reduces 100 PFU to 50 PFU.

TABLE 4. Persistence of LCM virus in blood and organs of β_2m-free mice lacking CD8+ T lymphocytes

Organ	Mouse	MIU[a] on days postinfection[b]:				
		5	15	30	50	100
Blood	−/−	$<1 \times 10^4$	1.04×10^6	5.76×10^6	1.16×10^6	$(1.29 \pm 0.77) \times 10^6$
	+/+	$<1 \times 10^4$	$<1 \times 10^4$	$<1 \times 10^4$	$<1 \times 10^4$	$<1 \times 10^4$
Spleen	−/−	3.01×10^9	2.99×10^8	1.64×10^8	2.94×10^8	$(2.78 \pm 1.40) \times 10^8$
	+/+	2.68×10^8	$\sim 4 \times 10^4$	$<1 \times 10^4$	$<1 \times 10^4$	$\sim 1 \times 10^5$
Liver	−/−	3.64×10^6	1.60×10^8	7.24×10^7	3.03×10^7	$(1.75 \pm 0.49) \times 10^7$
	+/+	2.22×10^6	$<1 \times 10^4$	$\sim 4 \times 10^4$	$<1 \times 10^4$	$\sim 9 \times 10^5$
Kidney	−/−	$<1 \times 10^4$	7.61×10^7	1.48×10^8	3.99×10^7	$(4.01 \pm 0.45) \times 10^7$
	+/+	$<1 \times 10^4$	$<1 \times 10^4$	$<1 \times 10^4$	$<1 \times 10^4$	$<1 \times 10^4$
Brain	−/−	$<1 \times 10^4$	2.69×10^7	1.53×10^8	3.16×10^7	$(8.49 \pm 7.97) \times 10^7$
	+/+	$<1 \times 10^4$	$<1 \times 10^4$	$\sim 2 \times 10^4$	$<1 \times 10^4$	$<1 \times 10^4$

[a] Mean MIU per milliliter or gram from two mice. The results 100 days after infection are mean ± SE from four mice.
[b] Infection was by i.v. inoculation of 10^3 MIU of WE strain LCM virus.

It is generally assumed that the lymphocytic choriomeningitis following i.c. infection is closely related to the inflammation following inoculation of the virus into the foot. If correct, the occurrence of these phenomena should be correlated. Although this prediction was verified by Fung-Leung et al. (17), no LCM virus-specific DTH was revealed by us in β_2m-deficient mice. This negative result is the more surprising since anti-LCM virus antibodies were readily formed, which is a strong indication for the functioning of CD4+ helper cells (1, 42). Furthermore, *L. monocytogenes*-specific DTH did develop, although to a somewhat lesser extent than in the β_2m +/+ mice. This difference makes sense, because optimal expression of the *L. monocytogenes*-specific DTH reaction has been shown to require cooperation of CD4+ and CD8+ T lymphocytes (21). At present we cannot offer an explanation of why the absence of β_2m results in inability to develop LCM virus-specific DTH. Apparently, in these mice the CD4+ T lymphocytes have lost normal functions or, as attested by the capacity to lyse virus-infected target cells (46), have acquired functions that they normally do not have. The display of unexpected properties is not a unique feature of the β_2m-lacking mice; other natural or artificially created mutants do the same (reviewed in reference 9).

The result that we consider most important is the ability and the inability of the mice of Fung-Leung et al. (17) and the mice studied by us, respectively, to control the infection. The lack of CD8+ T lymphocytes in mice of the two strains has quite dissimilar reasons. Zijlstra et al. disrupted the gene for β_2m (61). Although not covalently bound to the heavy chain, this protein plays a role in the conformation of the class I complex and perhaps its expression on the cell surface (2, 24, 60). Consequently, few if any class I molecules are expressed on cells (35, 60), and this, in turn, prevents the development of CD8+ T lymphocytes (60). Hence, these mice not only are devoid of CD8+ cells but also cannot present antigen in association with class I molecules. Fung-Leung et al. disrupted the gene encoding Lyt-2, with the consequence that the CD8 heterodimer was not formed (18); antigen presentation, however, should not be impeded. Our explanation for the ability of these animals to eliminate the virus is that a subset of T lymphocytes which, under ordinary conditions, would have developed into CD8+ CTL is activated. These double-negative T cells cannot lyse virus-infected target cells, although they have retained other properties of antiviral effectors. We hasten to stress that this is currently a working hypothesis. The failure of Fung-Leung et al. (18) to detect CD3+ CD4− cells in their mice does not necessarily contradict it; one MAb (145-2C11) was used, and some structural alteration of CD3 due to the absence of CD8 might have prevented binding.

CD8 (as CD4) is generally assumed to increase the avidity between the T-cell receptor and its ligand (reviewed in references 4 and 40), which in this particular case of an *H-2*^{b} mouse is predominantly D^b plus a nonapeptide derived from the viral glycoprotein (48, 58). Both CD4 and CD8 are also involved in signal transduction (reviewed in reference 4). Our assumption that despite the absence of CD8+ T cells, mice generate MHC-restricted antiviral effector cells requires that selection and maturation of T lymphocytes within the thymus, as well as their activation and development in the periphery, happen without CD8. There is a considerable amount of literature on thymic education (reviewed in references 5, 16, 47, 50, and 55), but evidence indicating that CD8 is required does not appear to be cogent. The same is true with regard to events occurring in the periphery (reviewed in reference 4).

The ability of the Lyt-2- and β_2m-deficient mice to cope and not to cope, respectively, with the LCM virus may have reasons unrelated to antigen presentation. When looking for an alternative explanation, one has to keep in mind that immunologically specific contact between target and CD8+ effector cells is obviously required for controlling the murine infection with LCM virus (15) and other viruses (38, 41). Since essentially all cells in the mouse may participate in the infectious process, including the ones that lack class II molecules (26; our unpublished observations), clearance by CD4+ cells is an implausible mechanism. The same is true with regard to antibodies, which the β_2m-free mice produce in large quantities, including antibodies with the capacity to neutralize the virus; however, they do not control the infection. Nor is it likely that gamma interferon (34, 57) or activated mononuclear phagocytes (30) play major roles. NK cell activity is impaired in the β_2m-lacking mice (35), but these cells are probably not involved in terminating the LCM virus infection (8, 56); furthermore, lack of β_2m has recently been shown to decrease the susceptibility of cells in vivo to NK cells (19). Thus, present knowledge does not permit us to implicate other elements of the immune system. However, such considerations cannot be generalized. For terminating the experimental infection of uncompromised mice with influenza virus, CD8+ T lymphocytes are essential. However, mice of the same type as used by us are quite capable of eliminating the influenza virus (14), although

other β_2m-deficient mice are relatively slow in achieving clearance (3). These mice, in turn, have no difficulty in controlling vaccinia virus even after inoculation of high infectious doses (52). Obviously, the mode with which higher organisms deal with viruses is not as simple as one would like.

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REFERENCES

1. **Ahmed, R., L. D. Butler, and L. Bhatti.** 1988. $T4^+$ helper cell function in vivo: differential requirement for induction of antiviral cytotoxic T-cell and antibody responses. J. Virol. **62:**2102–2106.
2. **Allen, H., J. Fraser, D. Flyer, S. Calvin, and R. Flavell.** 1986. β_2-Microglobulin is not required for cell surface expression of the murine class I histocompatibility antigen $H\text{-}2D^b$ or of a truncated $H\text{-}2D^b$. Proc. Natl. Acad. Sci. USA **83:**7447–7451.
3. **Bender, B. S., T. Croghan, L. Zhang, and P. A. Small.** 1992. Transgenic mice lacking class I major histocompatibility complex-restricted T cells have delayed viral clearance and increased mortality after influenza virus challenge. J. Exp. Med. **175:**1143–1145.
4. **Bierer, B. E., B. P. Sleckman, S. E. Ratnofsky, and S. J. Burakoff.** 1989. The biologic roles of CD2, CD4, and CD8 in T-cell activation. Annu. Rev. Immunol. **7:**579–599.
5. **Blackman, M., J. Kappler, and P. Marrack.** 1990. The role of the T cell receptor in positive and negative selection of developing T cells. Science **248:**1335–1341.
6. **Brunner, K. T., J. Mauel, J.-C. Cerottini, and B. Chapuis.** 1968. Quantitative assay of the lytic action of immune lymphoid cells on ^{51}Cr-labelled allogeneic target cells *in vitro*: inhibition by isoantibody and by drugs. Immunology **14:**181–196.
7. **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:**275–331.
8. **Bukowski, J. F., J. F. Warner, G. Dennert, and R. M. Welsh.** 1985. Adoptive transfer studies demonstrating the antiviral effect of natural killer cells in vivo. J. Exp. Med. **161:**40–52.
9. **Davies, K.** 1992. Mulling over mouse models. Nature (London) **359:**86.
10. **Dialynas, D. P., D. B. Wilde, P. Marrack, A. Pierres, K. A. Wall, W. Havran, G. Otten, M. R. Loken, M. Pierres, J. Kappler, and F. W. Fitch.** 1983. Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen-reactivity. Immunol. Rev. **74:**29–56.
11. **Doherty, P. C., J. E. Allan, and R. Ceredig.** 1988. Contributions of host and donor T cells to the inflammatory process in murine lymphocytic choriomeningitis. Cell. Immunol. **116:**475–481.
12. **Doherty, P. C., J. E. Allan, F. Lynch, and R. Ceredig.** 1990. Dissection of an inflammatory process induced by $CD8^+$ T cells. Immunol. Today **11:**55–59.
13. **Doherty, P. C., and R. M. Zinkernagel.** 1974. T-cell-mediated immunopathology in viral infections. Transplant. Rev. **19:**89–120.
14. **Eichelberger, M., W. Allan, M. Zijlstra, R. Jaenisch, and P. C. Doherty.** 1991. Clearance of influenza virus respiratory infection in mice lacking class I major histocompatibility complex-restricted $CD8^+$ T cells. J. Exp. Med. **174:**875–880.
15. **Fang, L., A. Gessner, K. Kühlcke, J. Gossmann, and F. Lehmann-Grube.** 1991. Antiviral immune responses of fully allogeneic irradiation bone marrow mouse chimeras. J. Immunol. **147:**3133–3138.
16. **Finkel, T. H., R. T. Kubo, and J. C. Cambier.** 1991. T-cell development and transmembrane signaling: changing biological responses through an unchanging receptor. Immunol. Today **12:**79–85.
17. **Fung-Leung, W.-P., T. M. Kündig, R. M. Zinkernagel, and T. W. Mak.** 1991. Immune response against lymphocytic choriomeningitis virus infection in mice without CD8 expression. J. Exp. Med. **174:**1425–1429.
18. **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:**443–449.
19. **Glas, R., K. Sturmhöfel, G. J. Hämmerling, K. Kärre, and H.-G. Ljunggren.** 1992. Restoration of a tumorigenic phenotype by β_2-microglobulin transfection of EL-4 mutant cells. J. Exp. Med. **175:**843–846.
20. **Hotchin, J.** 1962. The foot pad reaction of mice to lymphocytic choriomeningitis virus. Virology **17:**214–216.
21. **Kaufmann, S. H. E., E. Hug, U. Väth, and I. Müller.** 1985. Effective protection against *Listeria monocytogenes* and delayed-type hypersensitivity to listerial antigens depend on cooperation between specific $L3T4^+$ and $Lyt\ 2^+$ T cells. Infect. Immun. **48:**263–266.
22. **Kennett, R. H.** 1980. Enzyme-linked antibody assay with cells attached to polyvinyl chloride plates, p. 376–377. *In* R. H. Kennett, T. J. McKearn, and K. B. Bechtol (ed.), Monoclonal antibodies. Hybridomas: a new dimension in biological analyses. Plenum Press, New York.
23. **Kimmig, W., and F. Lehmann-Grube.** 1979. The immune response of the mouse to lymphocytic choriomeningitis virus. I. Circulating antibodies. J. Gen. Virol. **45:**703–710.
24. **Krangel, M. S., H. T. Orr, and J. L. Strominger.** 1979. Assembly and maturation of HLA-A and HLA-B antigens in vivo. Cell **18:**979–991.
25. **Ledbetter, J. A., and L. A. Herzenberg.** 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol. Rev. **47:**63–90.

25a. **Lehmann-Grube, F.** Unpublished data.

26. **Lehmann-Grube, F.** 1971. Lymphocytic choriomeningitis virus. Virol. Monogr. **10:**1–173.
27. **Lehmann-Grube, F.** 1984. Portraits of viruses: arenaviruses. Intervirology **22:**121–145.
28. **Lehmann-Grube, F.** 1988. Mechanism of recovery from acute virus infection. VI. Replication of lymphocytic choriomeningitis virus in and clearance from the foot of the mouse. J. Gen. Virol. **69:**1883–1891.
29. **Lehmann-Grube, F., and J. Ambrassat.** 1977. A new method to detect lymphocytic choriomeningitis virus-specific antibody in human sera. J. Gen. Virol. **37:**85–92.
30. **Lehmann-Grube, F., I. Krenz, T. Krahnert, R. Schwachenwald, D. Moskophidis, J. Löhler, and C. J. Villeda Posada.** 1987. Mechanism of recovery from acute virus infection. IV. Questionable role of mononuclear phagocytes in the clearance of lymphocytic choriomeningitis virus from spleens of mice. J. Immunol. **138:**2282–2289.
31. **Lehmann-Grube, F., and J. Löhler.** 1981. Immunopathologic alterations of lymphatic tissues of mice infected with lymphocytic choriomeningitis virus. II. Pathogenetic mechanism. Lab. Invest. **44:**205–213.
32. **Lehmann-Grube, F., L. Martínez Peralta, M. Bruns, and J. Löhler.** 1983. Persistent infection of mice with the lymphocytic choriomeningitis virus. Compr. Virol. **18:**43–103.
33. **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. N.Y. Acad. Sci. **532**:238–256.
34. **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**:2813–2819.
35. **Liao, N.-S., M. Bix, M. Zijlstra, R. Jaenisch, and D. Raulet.** 1991. MHC class I deficiency: susceptibility to natural killer (NK) cells and impaired NK activity. Science **253**:199–202.
36. **Löhler, J., J. Gossmann, T. Kratzberg, and F. Lehmann-Grube.** Murine hepatitis caused by lymphocytic choriomeningitis virus. I. Morphological analysis of the hepatic lesions. Submitted for publication.
37. **Löhler, J., and F. Lehmann-Grube.** 1981. Immunopathologic alterations of lymphatic tissues of mice infected with lymphocytic choriomeningitis virus. I. Histopathologic findings. Lab. Invest. **44**:193–204.
38. **Lukacher, A. E., V. L. Braciale, and T. J. Braciale.** 1984. In vivo effector function of influenza virus-specific cytotoxic T lymphocyte clones is highly specific. J. Exp. Med. **160**:814–826.
39. **Mackaness, G. B.** 1962. Cellular resistance to infection. J. Exp. Med. **116**:381–406.
40. **Maryanski, J. L., P. Pala, J.-C. Cerottini, and H. R. MacDonald.** 1988. Antigen recognition by H-2-restricted cytolytic T lymphocytes: inhibition of cytolysis by anti-CD8 monoclonal antibodies depends upon both concentration and primary sequence of peptide antigen. Eur. J. Immunol. **18**:1863–1866.
41. **McIntyre, K. W., J. F. Bukowski, and R. M. Welsh.** 1985. Exquisite specificity of adoptive immunization in arenavirus-infected mice. Antiviral Res. **5**:299–305.
42. **Moskophidis, D., S. P. Cobbold, H. Waldmann, and F. Lehmann-Grube.** 1987. Mechanism of recovery from acute virus infection: treatment of lymphocytic choriomeningitis virus-infected mice with monoclonal antibodies reveals that Lyt-2+ T lymphocytes mediate clearance of virus and regulate the antiviral antibody response. J. Virol. **61**:1867–1874.
43. **Moskophidis, D., L. Fang, R. Drjupin, J. Löhler, M. Bruns, and F. Lehmann-Grube.** 1990. Virus-specific delayed-type hypersensitivity (DTH). Cells mediating lymphocytic choriomeningitis virus-specific DTH reaction in mice. J. Immunol. **144**:1926–1934.
44. **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**:3305–3316.
45. **Moskophidis, D., and F. Lehmann-Grube.** 1984. The immune response of the mouse to lymphocytic choriomeningitis virus. IV. Enumeration of antibody-producing cells in spleens during acute and persistent infection. J. Immunol. **133**:3366–3370.
46. **Muller, D., B. H. Koller, J. L. Whitton, K. E. LaPan, K. K. Brigman, and J. A. Frelinger.** 1992. LCMV-specific, class II-restricted cytotoxic T cells in β_2-microglobulin-deficient mice. Science **255**:1576–1578.
47. **Nikolić Žugić, J.** 1991. Phenotypic and functional stages in the intrathymic development of $\alpha\beta$ T cells. Immunol. Today **12**:65–70.
48. **Oldstone, M. B. A., J. L. Whitton, H. Lewicki, and A. Tishon.** 1988. Fine dissection of a nine amino acid glycoprotein epitope, a major determinant recognized by lymphocytic choriomeningitis virus-specific class I-restricted H-2D^b cytotoxic T lymphocytes. J. Exp. Med. **168**:559–570.
49. **Qin, S., S. Cobbold, H. Tighe, R. Benjamin, and H. Waldmann.** 1987. CD4 monoclonal antibody pairs for immunosuppression and tolerance induction. Eur. J. Immunol. **17**:1159–1165.
50. **Ritter, M. A., and I. N. Crispe.** 1992. The thymus. Oxford University Press, Oxford.
51. **Rivers, T. M., and T. F. M. Scott.** 1935. Meningitis in man caused by a filterable virus. Science **81**:439–440.
52. **Spriggs, M. K., B. H. Koller, T. Sato, P. J. Morrissey, W. C. Fanslow, O. Smithies, R. F. Voice, M. B. Widmer, and C. R. Maliszewski.** 1992. β_2-Microglobulin$^-$, CD8$^+$ T-cell-deficient mice survive inoculation with high doses of vaccinia virus and exhibit altered IgG responses. Proc. Natl. Acad. Sci. USA **89**:6070–6074.
53. **Thomsen, A. R., and O. Marker.** 1989. MHC and non-MHC genes regulate elimination of lymphocytic choriomeningitis virus and antiviral cytotoxic T lymphocyte and delayed-type hypersensitivity mediating T lymphocyte activity in parallel. J. Immunol. **142**:1333–1341.
54. **Volkert, M., and J. Hannover Larsen.** 1965. Immunological tolerance to viruses. Prog. Med. Virol. **7**:160–207.
55. **Von Boehmer, H., and P. Kisielow.** 1990. Self-nonself discrimination by T cells. Science **248**:1369–1373.
56. **Welsh, R. M., J. O. Brubaker, M. Vargas-Cortes, and C. L. O'Connell.** 1991. Natural killer (NK) cell response to virus infections in mice with severe combined immunodeficiency. The stimulation of NK cells and the NK cell-dependent control of virus infections occur independently of T and B cell function. J. Exp. Med. **173**:1053–1063.
57. **Wille, A., A. Gessner, H. Lother, and F. Lehmann-Grube.** 1989. Mechanism of recovery from acute virus infection. VIII. Treatment of lymphocytic choriomeningitis virus-infected mice with anti-interferon-γ monoclonal antibody blocks generation of virus-specific cytotoxic T lymphocytes and virus elimination. Eur. J. Immunol. **19**:1283–1288.
58. **Yanagi, Y., A. Tishon, H. Lewicki, B. A. Cubitt, and M. B. A. Oldstone.** 1992. Diversity of T-cell receptors in virus-specific cytotoxic T lymphocytes recognizing three distinct viral epitopes restricted by a single major histocompatibility complex molecule. J. Virol. **66**:2527–2531.
59. **Zijlstra, M., H. Auchincloss, J. M. Loring, C. M. Chase, P. S. Russell, and R. Jaenisch.** 1992. Skin graft rejection by β_2-microglobulin-deficient mice. J. Exp. Med. **175**:885–893.
60. **Zijlstra, M., M. Bix, N. E. Simister, J. M. Loring, D. H. Raulet, and R. Jaenisch.** 1990. β2-microglobulin deficient mice lack CD4$^-$8$^+$ cytolytic T cells. Nature (London) **344**:742–746.
61. **Zijlstra, M., E. Li, F. Sajjadi, S. Subramani, and R. Jaenisch.** 1989. Germ-line transmission of a disrupted β_2-microglobulin gene produced by homologous recombination in embryonic stem cells. Nature (London) **342**:435–438.
62. **Zinkernagel, R. M., and P. C. Doherty.** 1979. MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function, and responsiveness. Adv. Immunol. **27**: 51–177.
63. **Zinkernagel, R. M., E. Haenseler, T. Leist, A. Cerny, H. Hengartner, and A. Althage.** 1986. T cell-mediated hepatitis in mice infected with lymphocytic choriomeningitis virus. Liver cell destruction by H-2 class I-restricted virus-specific cytotoxic T cells as a physiological correlate of the ^{51}Cr-release assay? J. Exp. Med. **164**:1075–1092.
64. **Zinkernagel, R. M., and R. M. Welsh.** 1976. 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**:1495–1502.