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Lymphotoxin-α- and Lymphotoxin-β-Deficient Mice Differ in Susceptibility to Scrapie: Evidence against Dendritic Cell Involvement in Neuroinvasion†

Michael B. A. Oldstone,[1]* Richard Race,[2] Diane Thomas,[1] Hanna Lewicki,[1] Dirk Homann,[1] Sara Smelt,[1] Andreas Holz,[1] Pandelakis Koni,[3] David Lo,[4] Bruce Chesebro,[2] and Richard Flavell[3]

Division of Virology, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California 92037[1]; Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton, Montana 59840[2]; Howard Hughes Medical Institute, Section of Immunobiology, Yale University School of Medicine, New Haven, Connecticut 06520[3]; and Digital Gene Technologies, Inc., La Jolla, California 92037[4]

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Transmissible spongiform encephalopathy or prion diseases are fatal neurodegenerative disorders of humans and animals often initiated by oral intake of an infectious agent. Current evidence suggests that infection occurs initially in the lymphoid tissues and subsequently in the central nervous system (CNS). The identity of infected lymphoid cells remains controversial, but recent studies point to the involvement of both follicular dendritic cells (FDC) and CD11c$^+$ lymphoid dendritic cells. FDC generation and maintenance in germinal centers is dependent on lymphotoxin alpha (LT-α) and LT-β signaling components. We report here that by the oral route, LT-α −/− mice developed scrapie while LT-β −/− mice did not. Furthermore, LT-α −/− mice had a higher incidence and shorter incubation period for developing disease following inoculation than did LT-β −/− mice. Transplantation of lymphoid tissues from LT-β −/− mice, which have cervical and mesenteric lymph nodes, into LT-α −/− mice, which do not, did not alter the incidence of CNS scrapie. In other studies, a virus that is tropic for and alters functions of CD11c$^+$ cells did not alter the kinetics of neuroinvasion of scrapie. Our results suggest that neither FDC nor CD11c$^+$ cells are essential for neuroinvasion after high doses of RML scrapie. Further, it is possible that an as yet unidentified cell found more abundantly in LT-α −/− than in LT-β −/− mice may assist in the amplification of scrapie infection in the periphery and favor susceptibility to CNS disease following peripheral routes of infection.

Transmissible spongiform encephalopathy (TSE), scrapie, or prion diseases are fatal neurodegenerative disorders (12, 30, 40). In many natural and experimental situations transmission occurs by ingestion of infectious material. In addition to transmission by ingestion, TSE disease can be transmitted by inoculation or transplantation of infected tissues or extracts, for example in iatrogenic human TSE or in experimental animal TSE models. Typically, TSE infection of humans and animals results in conversion of normal prion protein (PrP^c) to the disease-associated protease-resistant form, PrP^{sc}. Follicular dendritic cells (FDCs) in germinal centers of lymphoid organs have been implicated as initial sites of accumulation of PrP^{sc} and infectivity (see reviews [12, 30, 40]). The subsequent passage of scrapie infection from peripheral lymphoid sites to the central nervous system (CNS) occurs mostly via neuronal transport (5, 18, 31, 34). This was shown in a variety of experiments using normal animals infected with scrapie in which the agent was tracked in nerves or failed to reach the CNS when nerves were cut. In other experiments neuroinvasion was seen after oral or intraperitoneal (i.p.) infection of mice in which PrP was expressed only in neurons using the neuron-specific enolase promoter, strongly suggesting that transmission is restricted by neurons (31). This conclusion was further supported as splenectomy did not influence the kinetics of migration and incidence of TSE disease, suggesting that FDCs did not play a role.

FDCs (8, 9, 17, 23, 25–27) and in recent reports CD11c$^+$ dendritic cells (3) appear to play an important role in the peripheral amplification of infectivity prior to neuroinvasion. In the present study we evaluated the role of FDCs in oral and i.p. infection of mice by scrapie agent. Since FDC generation and maintenance in germinal centers is dependent on lymphotoxin alpha (LT-α) and LT-β signaling components (2, 4, 13, 15, 20, 39), we infected LT-α and LT-β knockout mice with scrapie and studied the accumulation of PrP^{sc} in their spleens and brains, the presence of infectious scrapie in their CNS, and the development of progressive neurodegenerative disease. We also investigated whether CD11c$^+$ dendritic cells played an essential role in neuroinvasion of scrapie by using lymphocytic choriomeningitis virus (LCMV) Clone 13, which specifically infects and aborts the function of such CD11c$^+$ cells (21, 35). Interestingly, LT-α$^{-/-}$ mice were found to have a higher disease incidence and shorter incubation period than LT-β$^{-/-}$ mice despite similar depletion of FDCs. Our results supported the conclusion that neither FDCs nor CD11c$^+$ cells are absolutely required for neuroinvasion in TSE disease caused by the RML strain of mouse scrapie. Further, our findings indicate that in addition to FDCs and CD11c$^+$ cells, a unique cell(s) found more abundantly in LT-α$^{-/-}$ than in LT-β$^{-/-}$ mice

* Corresponding author. Mailing address: Department of Neuropharmacology (IMM-6), The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037. Phone: (858) 784-8054. Fax: (858) 784-9981. E-mail: mbaobo@scripps.edu.

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likely assists in the amplification of scrapie infection in the periphery and favors susceptibility for CNS disease following peripheral routes of infection.

MATERIALS AND METHODS

Mice. LT-$\beta^{-/-}$ mice and LT-$\beta^{+/+}$ mice were described previously (20). LT-$\alpha^{-/-}$ mice were derived originally by David Chaplin (Washington University, St. Louis, Mo.) (14). $RelB^{-/-}$ mice were made as described previously (10). Genotyping confirmed their LT-α and LT-β status (10, 14, 20). All mice were on a mixed background of C57Bl/6 and 129/Sv (B6 × 129). CD4, CD8, β2-microglobulin, and major histocompatibility complex (MHC) class II gene-disrupted mice on B6 background were derived and characterized as reported. Breeding and maintenance of these mice and of C57Bl/6 and BALB/cjd mice were performed under specific-pathogen-free conditions at the Rodent Breeding Colony, The Scripps Research Institute (La Jolla, Calif.).

Infectious agents and infection of mice. Mice were inoculated by oral, i.p., or intracerebral (i.c.) routes with various doses of the RML strain of mouse scrapie (31). i.p. inoculation volume was 100 λ, and i.c. inoculation volume was 30 λ. Oral infection was in a volume of 200 λ administered via a small-diameter flexible polypropylene catheter inserted over the base of the tongue about 1 to 2 cm into the esophagus (31). Quantitation of infectious scrapie in brains was accomplished by i.c. injection of serial 10-fold dilutions of a 10% brain homogenate into B6 mice (four mice/dilution). The LCMV ARM 53b strain is a triple plaque-purified isolate of ARM CA 1371 and was passaged in baby hamster kidney (BHK) cells (35). Cl 13 is a triple plaque-purified variant of ARM 53b derived from spleen cells of an adult BALB/WEHI mouse persistently infected since birth with ARM 53b (1, 33). Either virus (2×10^6 PFU) was inoculated intravenously (i.v.) into B6 × 129, B6, or BALB/cdj mice (35). Inoculated mice were sacrificed when moribund, or when healthy at 11 to 12 months postadministration of mouse scrapie.

RNase protection assay. The RNase protection assay was performed using probe sets from Pharmingen (La Jolla, Calif.) where 20 μg of total RNA obtained from brains of control, LT-$\alpha^{-/-}$, LT-$\beta^{-/-}$, or $RelB^{-/-}$ mice were hybridized to ^{32}P-labeled riboprobes from various sets (16). After digestion with RNase, the material was separated on a sequencing gel, and the intensity of the protected RNA fragments was quantified using an ImageQuant system with appropriate software (Molecular Dynamics). Each transcript level was normalized to the ubiquitous housekeeping L32 RNA.

PrP^{sc} purification. Twenty-percent tissue homogenates were made in a solution containing 0.01 M Tris-HCl (pH 7.4) and 0.005 M MgCl using disposable Konex microcentrifuge tubes and matched pestles. Homogenates were sonicated for 2 min, and then DNase was added (1.0 mg/g of starting tissue) and the suspension incubated for 1 h at 37°C in a swirling water bath. An equal volume of 20% sarcosyl in 0.01 M Tris-HCl (pH 7.4) was added, and the suspension was vortexed and then incubated at room temperature for 1 h. Suspensions were centrifuged at 10,000 × g, 30 min, 10°C, and the resulting supernatant was centrifuged at 215,000 × g for 2 h at 10°C. The resulting pellet was resuspended by sonication in sterile water (1.0 ml/200 mg of starting tissue). Twenty-five micrograms of proteinase K/200 mg of starting tissue was added, and the suspension was incubated at 37°C in a swirling water bath for 30 min. Proteinase K activity was terminated by the addition of 25 μl of 0.1 M phenylmethylsulfonyl fluoride/ml of suspension followed by cooling on ice for 15 min. Suspensions were then centrifuged at 215,000 × g for 1 h at 10°C. Pellets were resuspended in sample buffer by sonication, boiled 5 min, and then frozen until needed. Immunoblotting as described previously (31) was used to detect PrP^{sc}.

RESULTS

Comparison of scrapie infection by various routes in LT-α and LT-β gene knockout mice. Injection of 10^7 50% lethal doses (LD_{50}) of the RML strain of murine scrapie directly into the brains of control (LT-$\alpha^{+/+}$ and LT-$\beta^{+/+}$), LT-$\alpha^{-/-}$, or LT-$\beta^{-/-}$ mice led to 100% mortality in all groups studied (four to six mice/group) with either death or severe morbidity requiring sacrifice at nearly equivalent times postinoculation (Fig. 1; Table 1). As expected, brains harvested from representative mice of each group expressed PrP^{sc} (Table 1; Fig. 2) and the classic histologic picture of neuronal degeneration, spongiosis, and astrocytosis. In preliminary studies normal control mice receiving $10^{5.5}$ LD_{50} orally did not develop TSE by 380 days, whereas all given 10^7 LD_{50} orally died before 290 days (five mice/group). Therefore, for all subsequent experiments 10^7 LD_{50} of mouse scrapie was administered orally or i.p. When control mice (10 per group) received murine scrapie orally or by the i.p. route with 10^7 LD_{50} of RML scrapie, all died by day 288 ± 8 (mean ± 1 standard deviation: oral route) or day 189 ± 5 (i.p. route), expressed PrP^{sc}, and manifested abnormal histopathology in their brains (Table 1). To the contrary and as anticipated, none of the $PrP^{-/-}$ mice inoculated i.c. became clinically ill over a 375-day observation period; these mice did not express PrP^{sc}, nor were histopathologic abnormalities evident in brain tissue.

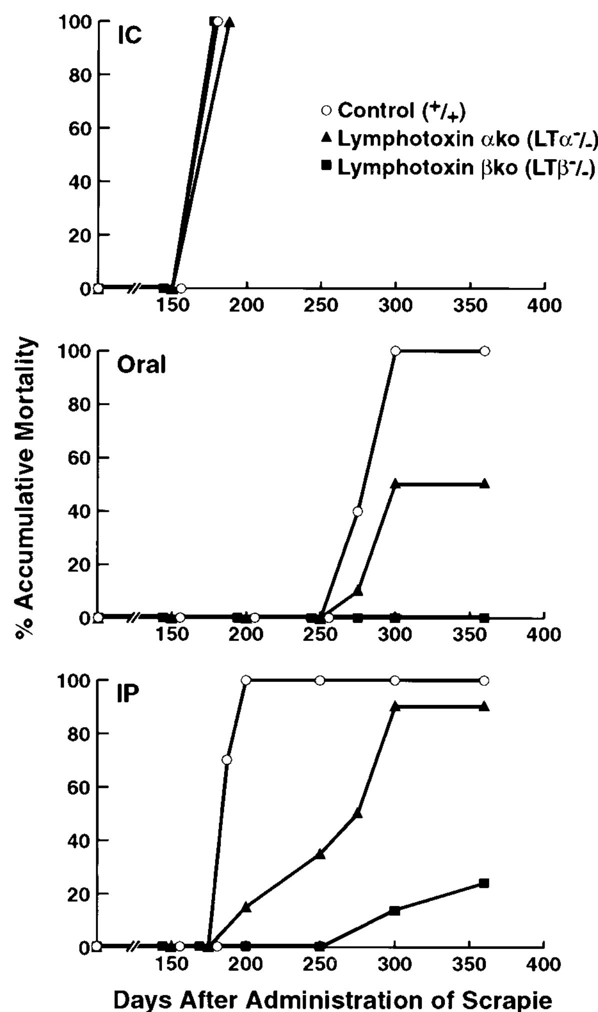

FIG. 1. LT-$\alpha^{-/-}$ mice and LT-$\beta^{-/-}$ mice differ in susceptibility to RML mouse scrapie administered orally or i.p. Control LT-$\alpha^{-/-}$ mice and LT-$\beta^{-/-}$ mice were 8 weeks old when inoculated. For i.c. inoculation, four control mice, six LT-$\alpha^{-/-}$ mice, and six LT-$\beta^{-/-}$ mice were used. For oral inoculation, 10 control mice, 10 LT-$\alpha^{-/-}$ mice, and 10 LT-$\beta^{-/-}$ mice were injected. Similar results occurred after a repeat experiment with six LT-$\alpha^{-/-}$ mice and six LT-$\beta^{-/-}$ mice. For i.p. inoculation, 10 control mice, 10 LT-$\alpha^{-/-}$ mice, and 16 LT-$\beta^{-/-}$ mice were employed.

None of the 10 LT-$\beta^{-/-}$ mice receiving 10^7 LD_{50} of RML scrapie by the oral route died over a 314- to a 365-day obser-

TABLE 1. Scrapie disease in LT-$\alpha^{-/-}$, LT-$\beta^{-/-}$, Rel$B^{-/-}$, and control mice[a]

Mouse genotype	Route of scrapie inoculation[b]	No. of mice developing disease[c]	Days (d) to terminal disease or sacrifice[d]	Detection of PrP^{sc} in CNS[c]
$PrP^{-/-}$	i.c.	0/3	375	0/3
$PrP^{+/+}$	i.c.	6/6	180 ± 4[e]	4/4
LT-$\alpha^{-/-}$	i.c.	4/4	189 ± 5[e]	4/4
LT-$\beta^{-/-}$	i.c.	4/4	178 ± 4[e]	4/4
Rel$B^{-/-}$	i.c.	2/2	179 ± 4[e]	2/2
$LT^{+/+}$	Oral	10/10	288 ± 8[e]	7/7
LT-$\alpha^{-/-}$	Oral	5/5	d275, d298, d278, d291, d285	5/5
		0/5	d345, d345, d360, d365, d365	0/5
LT-$\beta^{-/-}$	Oral	0/10	d314, d314, d349, d349, d349, d349, d360, d360, d365, d365	0/10
Rel$B^{+/+}$	Oral	2/2	d291, d296	2/2
Rel$B^{-/-}$	Oral	0/3	d365, d365, d365	0/3
$LT^{+/+}$	i.p.	10/10	189 ± 5[e]	4/4
LT-$\alpha^{-/-}$	i.p.	9/10	d211, d239, d239, d220, d220, d220, d247, d242, d242	8/8
		0/1	d348	1/1
LT-$\beta^{-/-}$	i.p.	5/5	d293, d293, d291, d299, d298	3/3
		0/11	d355, d355, d355, d355, d355, d360, d360, d368, d368, d368, d368	0/9

[a] RML mouse scrapie (10^7 LD_{50}) was given to 8-week-old C57BL/6 × 129 mice.
[b] i.c., intracerebral; i.p., intraperitoneal.
[c] Number of mice positive/total mice in a group.
[d] Day mice were moribund or died, or they were sacrificed when clinically free of scrapie disease.
[e] Mean ± 1 standard deviation.

vation period (Fig. 1; Table 1). Neither infectivity nor PrP^{sc} was found in the brain of any of these orally inoculated LT-$\beta^{-/-}$ mice (Fig. 2; Table 1). We reported that tumor necrosis factor (TNF) mRNA was markedly increased in the brains of mice undergoing scrapie-induced disease, with the level of these transcripts paralleling the severity of disease (11). Accordingly, brains of LT-$\beta^{-/-}$ mice inoculated orally failed to overexpress either tumor necrosis factor alpha (TNF-α) or TNF-β mRNA (Fig. 3).

LT-$\alpha^{-/-}$ mice inoculated orally segregated into two groups. Fifty percent developed clinical scrapie and were sacrificed by day 285 ± 4, whereas the other 50% failed to show clinical disease by day 345 to 360 (Fig. 1; Table 1). As anticipated, those LT-$\alpha^{-/-}$ mice developing clinical scrapie expressed both PrP^{sc} (Fig. 2) and TNF mRNA (Fig. 3) in their brains. Furthermore, infectivity was found in the brains of these mice as detected by inoculation into normal recipients.

The differences between LT-$\beta^{-/-}$ and LT-$\alpha^{-/-}$ mice as regards susceptibility to scrapie infection were further confirmed when these mice were given scrapie by the i.p. route. In this instance, 9 of 10 inoculated LT-$\alpha^{-/-}$ mice developed clinical scrapie and had PrP^{sc} in their brains. The onset of disease was significantly ($P < 0.01$) slower (230 ± 5 days) in these LT-$\alpha^{-/-}$ mice than in age- and sex-matched LT-$\alpha^{+/+}$ mice (day 189 ± 5) given the same dose of scrapie. The lone clinically healthy LT-$\alpha^{-/-}$ mouse was sacrificed at 348 days after scrapie inoculation, and its brain contained PrP^{sc} and infectious scrapie. In contrast to LT-$\alpha^{-/-}$ mice, only 5 of 16 (31%) LT-$\beta^{-/-}$ mice developed TSE disease after i.p. inoculation of scrapie and had PrP^{sc} in their brains, whereas the remaining 11 of 16 (69%) mice did not (Fig. 1 and 2; Table 1). Brains from the latter group also failed to express TNF mRNA (Fig. 3). The mean time interval required for disease to develop was shorter in LT-$\alpha^{-/-}$ mice (day 230 ± 5) than in the LT-$\beta^{-/-}$ group (day 295 ± 2) ($P < 0.001$). Intracerebral inoculation of 10% brain homogenates from five of the i.p.-inoculated clinically healthy, PrP^{sc} nil LT-$\beta^{-/-}$ mice into control mice has not led to scrapie over a 345-day observation period.

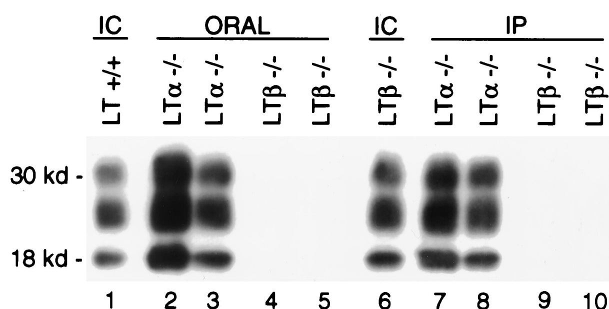

FIG. 2. Lack of PrP^{sc} expression in brains of LT-$\beta^{-/-}$ mice inoculated orally or i.p. with 10^7 LD_{50} of RML mouse scrapie (see Table 1). Lanes 2 and 3 represent two LT-$\alpha^{-/-}$ mice inoculated orally, and lanes 7 and 8 show results for two other LT-$\alpha^{-/-}$ mice inoculated i.p. All illustrate the expression of PrP^{sc}. As displayed in lanes 4 and 5, no expression of PrP^{sc} was evident in brains of LT-$\beta^{-/-}$ mice after oral inoculation or after i.p. inoculation, as in lanes 9 and 10. Similar results were found with additional samples from LT-$\alpha^{-/-}$ and LT-$\beta^{-/-}$ mice as recorded in Table 1.

Enhanced susceptibility of LT-$\alpha^{-/-}$ mice over LT-$\beta^{-/-}$ mice is not due to differences in FDC populations of LT-$\alpha^{-/-}$ or LT-$\beta^{-/-}$ mice or the presence of cervical and mesenteric lymph nodes or thymic tissues in LT-$\beta^{-/-}$ mice. The enhanced susceptibility of LT-$\alpha^{-/-}$ mice over that of LT-$\beta^{-/-}$ mice to both oral and i.p. administration of scrapie, coupled with the longer interval between infection and clinical disease in LT-

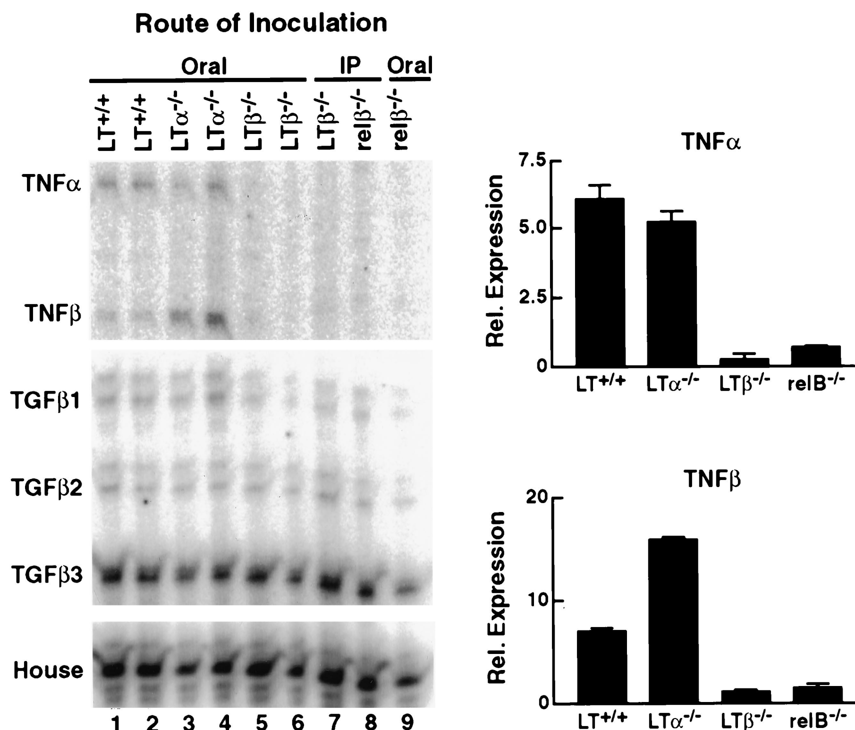

FIG. 3. Transcripts of TNF-α or -β mRNA appear in brains of LT-$\alpha^{-/-}$ mice receiving 10^7 LD_{50} of RML scrapie orally or i.p. These mice develop clinical disease, display histopathologic lesions, and express PrP^{sc} associated with scrapie disease. In contrast, LT-$\beta^{-/-}$ mice after oral or i.p. inoculation, or RelB−/− mice after oral administration of scrapie, fail to develop scrapie disease or show transcripts of TNF-α or -β mRNA in their brains. Results were similar for three additional LT-$\beta^{-/-}$ mice and one $RelB^{-/-}$ mouse studied (data not shown). Each transcript level was normalized to the ubiquitous housekeeping L32 RNA.

$\beta^{-/-}$ mice (day 295 ± 2) compared to LT-$\alpha^{-/-}$ mice (day 230 ± 5), led us to evaluate both the numbers of FDCs in LT-$\beta^{-/-}$ mice versus LT-$\alpha^{-/-}$ mice and possible immunologic protection by cervical and mesenteric lymph nodes or thymic tissues in LT-$\beta^{-/-}$ mice. FDCs are known cells in lymphoid organs implicated in the replication of scrapie infectivity (8, 9, 17, 23, 25–27). Examination of three spleens randomly chosen from both LT-$\alpha^{-/-}$ and LT-$\beta^{-/-}$ mice, as expected from observations of others (2, 10, 13, 14, 20), showed the absence of FDCs as determined by the use of FDC-M2 antibodies on cryomicrotome sections. Hence, by the assays used, the susceptibility of LT-$\alpha^{-/-}$ mice to peripheral routes of scrapie infection was not attributable to residual or extra FDCs.

LT-$\beta^{-/-}$ mice are reported to have cervical and mesenteric lymph nodes, whereas LT-$\alpha^{-/-}$ mice do not (2, 14, 20, 22). However, one report (4) concluded that a subset of LT-$\alpha^{-/-}$ mice have abnormal lymphoid-like structures in their mesenteric fat, perhaps accounting for the biphasic response we noted (Fig. 1). To ascertain whether lymphoid cells might provide resistance against peripheral TSE infection for LT-$\beta^{-/-}$ mice, we removed their cervical and mesenteric lymph nodes and initially examined such tissues for FDCs using FDC-M2 antibodies. As anticipated, no FDCs were observed. In a separate series of studies we adoptively transferred cervical and mesenteric lymph node cells i.p. or implanted the tissue with and without thymic tissue from LT-$\beta^{-/-}$ mice (four mice/group) under the renal capsule (38) of LT-$\alpha^{-/-}$ mice. One week after transplantation, LT-$\alpha^{-/-}$ mice reconstituted with LT-β lymphoid cells were challenged with 10^7 LD_{50} of RML scrapie i.p. No difference was seen in the time required for developing scrapie in LT-$\alpha^{-/-}$ mice reconstituted with LT-β lymphoid cells (days 230 to 250) compared to regular LT-$\alpha^{-/-}$ mice. These results indicated it is likely that the cervical and mesenteric lymph nodes and thymic tissue from LT-$\beta^{-/-}$ mice had no protective effect against TSE disease.

$RelB^{-/-}$ mice fail to develop TSE after oral administration of mouse scrapie. $RelB^{-/-}$ mice, like LT-$\alpha^{-/-}$ and LT-$\beta^{-/-}$ mice, lack FDCs and exhibit disordered splenic architecture (10). However, unlike LT-$\alpha^{-/-}$ or LT-$\beta^{-/-}$ mice, $RelB^{-/-}$ mice also have abnormal thymic medullary cells. $RelB^{-/-}$ mice given scrapie orally failed to develop disease and did not express PrP^{sc} (Table 1) or TNF mRNA in their brains (Fig. 3) over a 1-year observation time (three of three mice). In contrast, i.c. inoculation of scrapie into $RelB^{-/-}$ mice resulted in both a clinical and a histopathologic picture of scrapie disease as well as expression of PrP^{sc} in brain tissues. Therefore, $RelB^{-/-}$ mice, like LT-$\beta^{-/-}$ mice, are resistant to orally administered RML scrapie. By contrast, 200 days after oral inoculation of scrapie into $RelB^{+/-}$ or $RelB^{+/+}$ mice (two to three mice per group), clinical and histopathologic evidence of scrapie as well as expression of PrP^{sc} in the CNS were clear.

Dysfunction of splenic $DEC205^+$ and $CD11c^+$ lymphoid dendritic cells does not alter the kinetics for development of TSE after peripheral administration of mouse scrapie. After i.v. administration of 2×10^6 PFU of LCMV Cl 13, >80% of $DEC205^+$ and $CD11c^+$ dendritic cells are infected (35),

TABLE 2. Study of peripheral scrapie infection in mice with dysfunction of splenic DEC205$^+$ and CD11c$^+$ lymphoid dendritic cells[a]

Groups inoculated with RML scrapie:	Infection of CD11c$^+$ and DEC205$^+$ cells[b]	Immunosuppression of[c]:		Incidence of TSE[d]	Day to clinical disease	Expression of TSE disease in CNS[e]
		CTL	Antibody			
Orally						
Nonviral infection	Nil	No	No	4/4 (100%)	d310 ± 5	3/3
LCMV Cl 13 infection	++++ (>80%)	++	++	4/4 (100%)	d305 ± 5	3/3
LCMV ARM infection	+ (<10%)	No	No	4/4 (100%)	d310 ± 5	3/3
i.p.						
Nonviral infection	Nil	No	No	6/6 (100%)	d268 ± 3	3/3
LCMV Cl 13 infection	++++ (>80%)	++	++	8/9 (89%)	d272 ± 4	3/3
LCMV ARM infection	+ (<10%)	No	No	6/6 (100%)	d269 ± 3)	3/3

[a] Six- to eight-week-old BALB mice were inoculated i.v. with 2×10^6 PFU of LCMV Cl 13 or LCMV ARM. Twelve days later these mice and a control uninfected group were given 10^7 LD_{50} of RML scrapie orally or i.p. Similar results occurred when B6 mice were inoculated with LCMV and scrapie.

[b] Numbers of CD11c$^+$ cells and DEC205$^+$ cells following either LCMV Cl 13 or LCMV ARM infection were determined as reported using dual labeling with antibodies to CD11c$^+$ or DEC205$^+$ proteins for cell surface marker detection and to LCMV nucleoprotein for expression of virus in cells' cytoplasm (35).

[c] Assays to detect immunosuppression of MHC-restricted CD8$^+$ cytotoxic T lymphocytes specific for LCMV, vaccinia virus, or herpes simplex virus at day 7 to 8 after infection or to detect antibodies to human immunoglobulin G or keyhole limpet hemocyanin at days 28, 45, 100, and 150 after inoculation. See references 7, 29, 35, and 37. ++, 10- to 30-fold decrease in immune response compared to control mice.

[d] Number of mice with TSE/number studied.

[e] Number of mice developing histologic evidence of CNS TSE disease, i.e., expressing PrP^{sc} or having infectious scrapie upon i.c. transfer to B6 mice/total number studied per group.

splenic architecture is disrupted (7, 21, 28, 35–37), replication occurs primarily in the splenic white pulp (7, 21, 35, 37), and a generalized immunosuppression lasting more than 6 months ensues (7, 35, 37) (unpublished data). By contrast, i.v. inoculation of 2×10^6 PFU of the parental LCMV strain Armstrong (ARM) (which differs by two amino acids from the LCMV Cl 13 variant) (33), yields only minimal infection of DEC205$^+$ and CD11c$^+$ (<10%) cells (35), replicates preferentially in the red pulp of the spleen (21, 35, 36), causes only minimal disorganization of the splenic architecture early in infection, and does not cause immunosuppression (7, 35, 37).

At 12 days after LCMV Cl 13 inoculation, when more than 80% of DEC205$^+$ and CD11c$^+$ cells are infected, immunosuppression is extensive and these dendritic cells cannot act as antigen-presenting cells (7, 35), LCMV-infected mice were given 10^7 LD_{50} of RML scrapie orally or i.p. As shown in Table 2 for both oral and i.p. administration of scrapie, mice with and without impairment of their DEC205$^+$ or CD11c$^+$ cells developed TSE disease at equivalent times and had PrP^{sc} and infectious scrapie in their brains. Because the spleen is known to be an important site of amplification and accumulation of scrapie infectivity in mice, we tested spleens of LT-$\alpha^{-/-}$ or LT-$\beta^{-/-}$ mice by Western blot analysis for PrP^{sc} and found all to be negative (three of three mice tested per group at 60 and 200 days after scrapie inoculation) in contrast to wild-type control mice and mice coinfected with LCMV and scrapie (data not shown). Overall, these studies suggest that a peripheral cell other than FDCs and interdigitating dendritic cells (DEC205$^+$, CD11c$^+$), and presumably not located in the spleen, might be involved in the amplification of PrP^{sc}.

Peripheral scrapie infection in mice with genetic inactivation of other genes involved in the lymphoid system does not alter the kinetics for development of TSE. We evaluated whether other lymphoid cells were involved in scrapie infection by using a panel of gene knockout mice. Equivalent patterns of scrapie infection were found in CD8, CD4, β2 microglobulin, and MHC class II knockout mice and wild-type control mice after oral administration of scrapie. Similarly, after i.p. scrapie administration the initiation and incidence of TSE disease were virtually identical with those for wild-type controls in β2 microglobulin and MHC class II knockout mice and, in agreement with the earlier report of Klein et al. (19), in CD8$^{-/-}$ and CD4$^{-/-}$ mice.

DISCUSSION

Our study focused primarily on the oral transmission of scrapie to the CNS. Using a battery of gene knockout mice, we noted several intriguing findings. Notably, LT-$\alpha^{-/-}$ mice devoid of FDCs undergo neuroinvasion and replication of infectious scrapie after oral inoculation. This neuroinvasion in LT-$\alpha^{-/-}$ mice also occurs after i.p. inoculation. A profound difference in susceptibility to the development of TSE disease occurs in LT-$\alpha^{-/-}$ compared to LT-$\beta^{-/-}$ mice. The differences in kinetics and susceptibility of TSE disease between LT-$\alpha^{-/-}$ and LT-$\beta^{-/-}$ mice, for both of which groups FDCs are deleted as observed by several investigators (2, 13, 14, 20) and reconfirmed here, suggest the likelihood that an additional, previously unidentified cell type may be important for replication of TSE infectivity. Last, neuroinvasion of scrapie occurred in mice in which the function of CD11c$^+$ cells was compromised.

Our results with LT-$\beta^{-/-}$ mice inoculated with scrapie i.p. differ slightly from those of Manuelidis et al. (24), who studied i.p. inoculation of LT-$\beta^{-/-}$ and LT-$\beta^{+/+}$ mice. These investigators utilized a different source of infectious TSE material, mouse-adapted human Creutzfeldt-Jakob disease, strain Fukuoka-2. After inoculation of 2,000 infectious units, the disease incidence was 100% but a significant increase in the incubation period in LT-$\beta^{-/-}$ mice was observed, whereas after 20 infectious units the incidence of disease was lower in LT-$\beta^{-/-}$ mice. In contrast, we used a very high dose of the RML strain of mouse scrapie and found a disease incidence of only 5 out of 16 after i.p. infection of LT-$\beta^{-/-}$ mice. The differences in incidence of disease observed with LT-$\beta^{-/-}$ mice in these experiments may be related to the TSE strains used. For example, the Fukuoka-2 strain may be less dependent on

replication in FDCs prior to neuroinvasion than is the RML strain.

Earlier studies detected PrP^{sc} in FDCs from spleens (8, 9, 17, 23, 25–27). However, in our experiments the differences between LT-$\alpha^{-/-}$ and LT-$\beta^{-/-}$ mice, for both of which FDCs are deleted, suggest that an additional, previously unidentified cell type is also likely important for replication of TSE infectivity. However, we cannot exclude, although unlikely, the possibility of a small subset of FDCs in LT-$\alpha^{-/-}$ mice that is not recognized by FDC-M2 antibodies and the possibility that this subset of FDCs is missing in LT-$\beta^{-/-}$ mice. Recently, Aucouturier et al. (3), using adoptive transfers, reported that $CD11c^+$ splenic dendritic cells from scrapie-infected mice were able to transmit TSE. Our experiments with LCMV Cl 13 and our inability to find PrP^{sc} in spleens of LT-$\alpha^{-/-}$ or LT-$\beta^{-/-}$ mice indicate that a cell in addition to $DEC205^+$ or $CD11c^+$ dendritic cells is likely involved. Further, comparing a panel of gene knockout mice suggests that CD4 or CD8 T cells or cells bearing MHC class II molecules are not likely the source of PrP^{sc} amplification. These results are further supported by our lymphoid cell transfer from LT-$\beta^{-/-}$ mice into LT-$\alpha^{-/-}$ mice, which failed to alter the kinetics of TSE infection of LT-$\alpha^{-/-}$ mice. Use of high doses of hamster scrapie administered orally or i.p. into mice in which hamster PrP expression was restricted to neurons using the neuron-specific enolase promoter (31, 32) showed that neuroinvasion can occur in the absence of PrP expression in FDCs or $CD11c^+$ cells. While passage of scrapie infection from the peripheral site to the CNS occurs via neuronal transport, FDCs (see review (40) and perhaps $CD11c^+$ dendritic cells play an important role in the amplification of infectivity prior to neuroinvasion, our data here coupled with an earlier report by Klein et al. (19) using TNF receptor $1^{-/-}$ mice devoid of FDCs suggest that an additional, yet-to-be-defined cell in the periphery can also replicate and amplify scrapie infectivity. Further, LT-$\alpha^{-/-}$ and LT-$\beta^{-/-}$ mice handle the RML scrapie agent in distinctive ways, suggesting that these models may be informative for the search of the unknown cell(s). Interestingly and in contrast to these studies with scrapie, LT-$\alpha^{-/-}$ mice and LT-$\beta^{-/-}$ mice have been reported to handle another infectious agent equivalently (6).

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ADDENDUM IN PROOF

Concurrent with this article's acceptance, Prinz et al. published similar results (M. Prinz, F. Montrasio, M. A. Klein, P. Schwarz, J. Priller, B. Odermatt, K. Pfeffer, and A. Aguzzi, Proc. Natl. Acad. Sci. USA **99:**919–924, 2002).

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