Impaired Prion Replication in Spleens of Mice Lacking Functional Follicular Dendritic Cells

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In scrapie-infected mice, prions are found associated with splenic but not circulating B and T lymphocytes and in the stroma, which contains follicular dendritic cells (FDCs). Formation and maintenance of mature FDCs require the presence of B cells expressing membrane-bound lymphotoxin-α/β. Treatment of mice with soluble lymphotoxin-β receptor results in the disappearance of mature FDCs from the spleen. We show that this treatment abolishes splenic prion accumulation and retards neuroinvasion after intraperitoneal scrapie inoculation. These data provide evidence that FDCs are the principal sites for prion replication in the spleen.

Replication of prions in the spleen and prion transfer from the periphery to the central nervous system (neuroinvasion) is impaired in certain forms of murine immunodeficiency (*1–4*), the presence of mature B lymphocytes being essential (*5*). In spleens of wild-type mice inoculated intraperitoneally (i.p.), infectivity is associated with B and T lymphocytes as well as with the stroma, but not with the pulp-derived non–B, non–T cell fraction or with circulating lymphocytes (*6*). Infectivity in the stroma is thought to reside in radiation-resistant, prion protein (PrP)–expressing postmitotic cells (*7–9*). A prime candidate is the FDC, because the pathological isoform of the prion protein, PrP^{Sc}, colocalizes with FDCs (*10–12*) and because chimeric mice harboring PrP-expressing but not PrP-deficient FDCs propagate prions after i.p. inoculation (*9*). Although PrP expression is essential for sustaining prion replication, B lymphocytes that lack PrP restore prion accumulation in spleen and neuroinvasion in severe combined immunodeficient (SCID), RAG-$1^{-/-}$, and μMT mice (*13*), indicating a requirement for cells dependent on B cells or their products, such as mature FDCs.

Tumor necrosis factor and lymphotoxin are necessary for FDC development (*14–16*). Membrane-bound lymphotoxin-α/β (LT-α/β) heterotrimers signal through the LTβ receptor (LTβR) (*17*) present on activated lymphocytes (*18*, *19*) to elicit development and maintenance of secondary lymphoid organs (*14*, *20*, *21*). Inhibition of the LT-α/β pathway with LTβR-immunoglobulin fusion protein (LTβR-Ig) (*22*) leads to the disappearance of functional FDCs and to disappearance of markers such as FDC-M1, FDC-M2, or CR1 within 1 day (*20*, *23*) and later to disruption of B cell follicles, modification of splenic marginal zone macrophages (*20*, *23*), and reduction of dendritic cell number (*24*).

We studied the effect of LTβR-Ig treatment on the pathogenesis of scrapie in mice treated with 300 μg LTβR-Ig either 1 week before or 1 week after i.p. prion inoculation (*25*). Depletion of mature FDCs was maintained by weekly injection of 100 μg of fusion protein until the seventh week (inclusive) after inoculation (*26*). Immunohistochemical examination of spleen sections 1, 2, and 4 weeks after inoculation with antibodies to FDC-M1 (Fig. 1A), FDC-M2, or CR1 receptor (*27*) revealed that FDC networks disappeared 1 week after treatment (*20*, *23*) and remained absent during the 4 weeks of observation. Some FDC-M1–positive cells (residual FDCs or tingible body macrophages that cross-react with the FDC-M1 antibody) (*28*) could still be detected. Splenic architecture was severely disturbed after prolonged LTβR-Ig treatment (*20*, *27*). PrP staining was virtually abolished after the treatment, implying that FDCs or cells dependent on them are those with the highest PrP expression level in the spleen (*11*) (Fig. 1B).

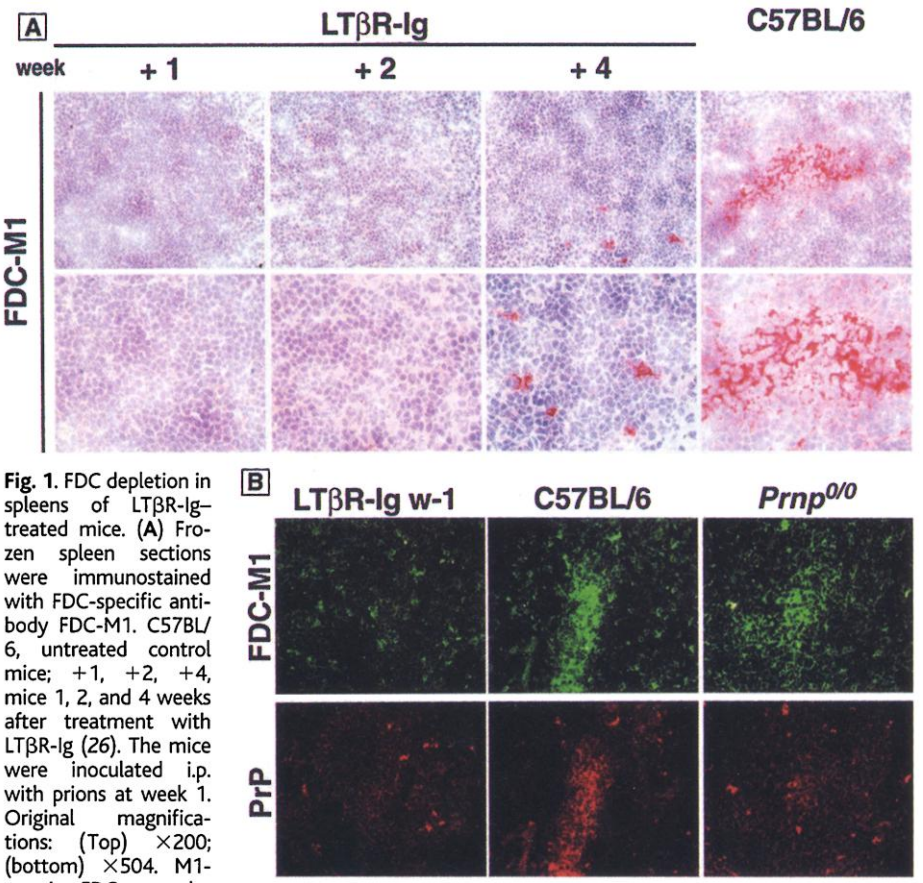

Fig. 1. FDC depletion in spleens of LTβR-Ig–treated mice. (**A**) Frozen spleen sections were immunostained with FDC-specific antibody FDC-M1. C57BL/6, untreated control mice; +1, +2, +4, mice 1, 2, and 4 weeks after treatment with LTβR-Ig (*26*). The mice were inoculated i.p. with prions at week 1. Original magnifications: (Top) ×200; (bottom) ×504. M1-reactive FDCs were depleted 1 week after treatment. Some FDC-M1–positive cells, either residual FDCs or tingible body macrophages, were still detected. (**B**) Double-color immunofluorescence analysis of splenic germinal centers. LTβR-Ig w-1, 1 week after treatment with LTβR-Ig; C57BL/6, untreated; *Prnp*$^{0/0}$, PrP knockout mice. Sections were stained with antibody FDC-M1 to FDCs (green, top) and with antiserum XN to PrP (red, bottom). The drastic decrease in PrP immunoreactivity after LTβR-Ig treatment shows that FDCs (or FDC-dependent cells) express the highest level of PrP in spleen. Original magnification, ×320.

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Table 1. Prion titers in spleens of LTβR-Ig–treated and control mice.

Mouse line	Treatment	Beginning of treatment*	Weeks after inoculation	Indicator mice succumbing to scrapie (unrelated causes)	Days to terminal disease	Titer†
*Prnp*0/0	–	–	1	0/4	>150	<1.5
*Prnp*0/0	–	–	1	0/1 (3)	>150	ND
C57BL/6	–	–	1	4/4	82, 91, 91, 113	3
C57BL/6	–	–	1	2/2 (2)	116, 128	1–1.5
C57BL/6	LTβR-Ig	−1	1	0/4	>150	<1.5
C57BL/6	LTβR-Ig	−1	1	1/4	101, >150; >150, >150	<1.5
C57BL/6	LTβR-Ig	−1	1	0/4	>150	<1.5
*Prnp*0/0	–	–	3	0/4	>150	<1.5
*Prnp*0/0	–	–	3	0/4	>150	<1.5
C57BL/6	huIgG	+1	3	0/4	>150	<1.5
C57BL/6	huIgG	+1	3	3/4	80, 91, 128, >150	1.5–2.5
C57BL/6	LTβR-Ig	−1	3	0/4	>150	<1.5
C57BL/6	LTβR-Ig	−1	3	0/4	>150	<1.5
C57BL/6	LTβR-Ig	−1	3	0/4	>150	<1.5
C57BL/6	LTβR-Ig	+1	3	3/4	87, 91, 136, >150	1.5–2
C57BL/6	LTβR-Ig	+1	3	4/4	80, 82, 84, 84	4
C57BL/6	LTβR-Ig	+1	3	1/4	108, >150, >150, >150	<1.5
*Prnp*0/0	–	–	8	0/4	>150	<1.5
*Prnp*0/0	–	–	8	0/4	>150	<1.5
C57BL/6	huIgG	+1	8	4/4	64, 68, 70, 70	5.5
C57BL/6	huIgG	+1	8	3/3 (1)	74, 80, 77	4.5
C57BL/6	LTβR-Ig	−1	8	0/4	>150	<1.5
C57BL/6	LTβR-Ig	−1	8	0/4	>150	<1.5
C57BL/6	LTβR-Ig	−1	8	0/4	>150	<1.5
C57BL/6	LTβR-Ig	+1	8	4/4	108, 110, 113, 144	1
C57BL/6	LTβR-Ig	+1	8	2/2 (2)	128, 144	1.5
C57BL/6	LTβR-Ig	+1	8	1/2 (2)	104, >150	ND

*Week relative to inoculation. †Spleen homogenates (10% in 0.32 M sucrose) were prepared from infected animals as described (*45*). Thirty microliters of heat- and sarcosyl-treated 1% homogenate (diluted 1:10 in PBS and 5% bovine serum albumin) were administered intracerebrally to groups of four *tga20* mice (*36*) for each sample. Wherever all indicator animals of a group contracted scrapie, titers (log ID_{50} units/ml 10% homogenate) were calculated by the incubation time method (*35*) using the relation $y = 11.45 - 0.088x$, where y is the ID_{50} and x is the incubation time (in days) to terminal disease. When one or more animals survived >150 days, the titer was assumed to be close to the end point; titers are approximate. ND, not determined.

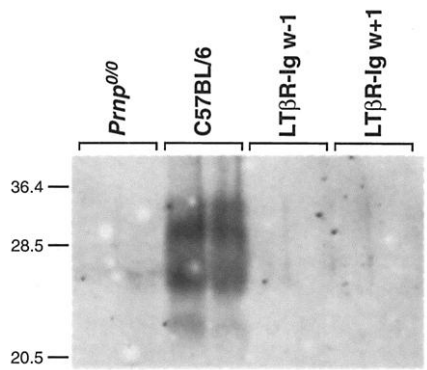

Fig. 2. Absence of PrP^{Sc} in spleens of LTβR-Ig–treated mice 8 weeks after inoculation. Immunoblot analysis of proteinase-K–treated spleen extracts (200 μg of total protein) from mice killed 8 weeks after i.p. inoculation. Spleens of two mice from each group were analyzed. *Prnp*$^{o/o}$, untreated C57BL/6 mice; LTβR-Ig w-1, C57BL/6 mice treated with LTβR-Ig starting 1 week before prion inoculation; LTβR-Ig w+1, treatment starting 1 week after inoculation. PrP was detected with a rabbit antiserum to murine PrP (1B3) (*44*) and enhanced chemiluminescence. The position of the molecular weight standards (in kilodaltons) is indicated on the left. LTβR-Ig treatment led to complete disappearance of the three bands diagnostic for PrP^{Sc} (less than 1/50th of untreated, wild-type controls).

PrP^{Sc} is detected in untreated spleen as early as 1 week after i.p. inoculation and persists throughout the disease (*29*, *30*). Likewise, infectivity appears within 7 days and reaches a plateau after 3 to 7 weeks (*31*–*33*). Western blots (Fig. 2) of spleen homogenates 8 weeks after i.p. inoculation (*34*) showed strong bands of protease-resistant PrP in control mice, whereas in mice injected weekly with LTβR-Ig, starting either 1 week before or 1 week after inoculation, no detectable signal appeared (less than 2% of controls).

Prion infectivity was assayed in three spleens for each time point (*35*–*37*) (Table 1). In spleens of mice treated with LTβR-Ig 1 week before i.p. inoculation, no infectivity was detected after 3 or 8 weeks [<1.5 log ID_{50} (50% infective dose) units per milliliter of 10% homogenate]. A trace of infectivity, possibly residual inoculum, was present in the 1-week samples. In three mice treated with LTβR-Ig 1 week after inoculation, the titers were <1.5, about 2 and 4 log ID_{50} units/ml 10% homogenate at 3 weeks, but only borderline infectivity was detected 8 weeks after infection, suggesting that some prion accumulation took place in the first week(s) after inoculation but was reversed under treatment with LTβR-Ig by 8 weeks. Spleens of scrapie-inoculated control mice treated with nonspecific human Ig (huIg) 1 week after inoculation and killed 8 weeks after inoculation had 4.5 and 5.5 log ID_{50} units/ml 10% homogenate. The low infectivity found in spleens of huIg-treated control mice 3 weeks after inoculation could be accidental or reflect inhibitory activity of the nonspecific huIg.

To assess whether prolonged depletion of mature FDCs delays neuroinvasion, mice were injected weekly with LTβR-Ig up to 8 weeks after inoculation and observed for >340 days (Table 2). Mice receiving LTβR-Ig starting 1 week after inoculation developed scrapie about 25 days later than control mice. When injections were initiated 1 week before inoculation, the effect was even more pronounced: Two of three mice developed scrapie symptoms 60 days later than huIg-treated controls, and one mouse survived >340 days (Table 2). Analysis of spleen sections of terminally sick animals revealed that the FDC networks were reconstituted after LTβR-Ig administration had been terminated 8 weeks after inoculation and that PrP colocalized with FDCs (Fig. 3A). Immunoblot analysis showed that PrP^{Sc} accumulation was restored, apparently concomitantly, with the reappearance of FDCs (Fig. 3B).

Table 2. Numbers of mice developing scrapie after i.p. inoculation.

Mouse line	Treatment	Beginning of treatment*	Mice succumbing to scrapie	Days to terminal disease (mean ± SD)
Prnp$^{0/0}$	–	–	0/5	–
C57BL/6	–	–	4/4	(206 ± 2)†
C57BL/6	hulg	+1	5/5	205, 211, 211, 214, 214 (211 ± 3)
C57BL/6	LTβR-Ig	+1	3/3	223, 223, 263 (236 ± 19)
C57BL/6	LTβR-Ig	−1	2/3	256, 256, >340

*Week relative to inoculation. †From (*13*).

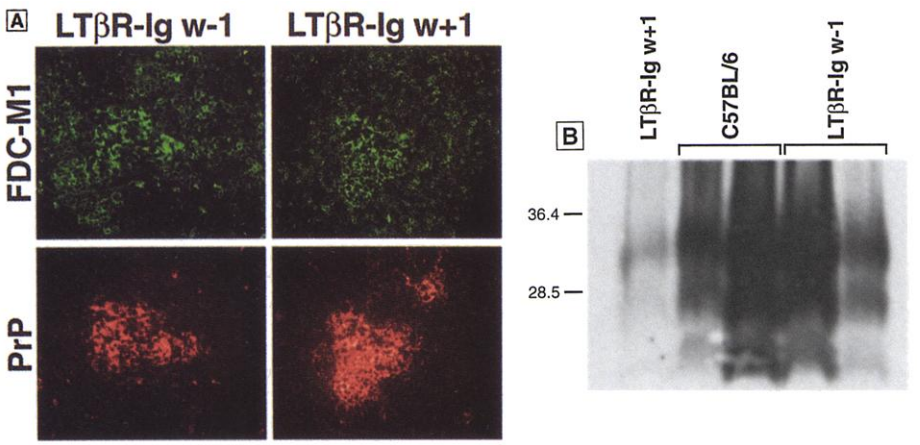

Fig. 3. Analysis of spleens from scrapie-inoculated mice treated with LTβR-Ig. Mice were treated with LTβR-Ig 1 week before (LTβR-Ig w-1) or after (LTβR-Ig w+1) scrapie inoculation (*26*). Terminally ill scrapie animals were culled (at about 220 to 260 days). (**A**) Double-color immunofluorescence analysis. Sections were stained with antibody FDC-M1 to FDCs (green, top) and with antiserum XN to PrP (red, bottom). Reappearance of M1-reactive FDCs is accompanied by strong PrP immunoreactivity, indicating that FDCs (or FDC-dependent cells) accumulate PrP. (**B**) Immunoblot analysis of spleen extracts (200 μg of total protein) from prion-inoculated LTβR-Ig–treated and control mice. Spleen homogenates were prepared and treated with proteinase K (*34*). Immunoreactive PrP was detected with polyclonal antibody 1B3 (*44*) and enhanced chemiluminescence. LTβR-Ig w+1, mice treated with LTβR-Ig 1 week after prion inoculation; C57BL/6, inoculated, untreated controls; LTβR-Ig w-1, mice treated 1 week before inoculation. Each lane represents results from an individual spleen. The position of the molecular weight standards (in kilodaltons) is indicated on the left. Reappearance of FDCs in the spleens of terminally sick LTβR-Ig-treated mice was accompanied by variable degrees of PrP^{Sc} accumulation.

Our results provide further evidence that FDCs are essential for accumulation of PrP^{Sc} and infectivity in the spleen and that they contribute, directly or indirectly, to neuroinvasion, complementing earlier conclusions based on immunocytochemical (*10*–*12*) and genetic approaches (*9*). To what extent, if any, the long-term effects of LTβR-Ig, such as the reduction of dendritic cell numbers (*24*) or marginal zone macrophages (*20*, *23*), affect scrapie pathogenesis in the spleen has not been assessed. The requirement for B cells for prion replication in the spleen and efficient neuroinvasion (*5*) is readily explained by their essential role in the maturation of FDCs. PrP knockout mice expressing PrP only in B cells do not sustain prion replication (*38*), suggesting that prions associated with splenic B cells (*6*) may be acquired from FDCs. The delay in neuroinvasion (*24*) caused by LTβR-Ig may be due either to the fact that prion propagation in the spleen was interrupted for some 8 weeks or that a pathway bypassing the lymphoreticular system altogether was used (*4*, *39*–*42*).

Because vCJD affects the lymphoreticular system before the appearance of clinical symptoms (*43*), one might speculate that early diagnosis and long-term treatment with LTβR-Ig could retard progression of the disease.

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26. One set of 15 C57BL/6 mice were injected i.p. with 300 μg of LTRβ-Ig (*19*) and challenged i.p. 1 week later with 7×10^4 LD_{50} units of mouse-adapted scrapie prions (RML 4.1). Depletion was maintained by weekly administration of 100 μg of the fusion protein for 8 weeks. A second set of mice were inoculated with prions as above; 300 μg of LTβR-Ig were injected 1 week later and 100 μg LTβR-Ig were injected once a week for a further 6 weeks. Three mice from each group were culled 1, 3, and 8 weeks after inoculation, respectively. As control, C57BL/6 mice were subjected to the same regime with a human IgG polyclonal preparation (hulgG, Sandoglobulin) instead of LTβR-Ig.
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