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NOTES

Species Barrier Prevents an Abnormal Isoform of Prion Protein from Accumulating in Follicular Dendritic Cells of Mice with Creutzfeldt-Jakob Disease

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The accumulation of abnormal prion protein in follicular dendritic cells did not occur in mice inoculated with materials from human Creutzfeldt-Jakob disease, whereas it always occurred in mice inoculated with mouse-adapted agents, suggesting an intense expression of the species barrier in the lymphoreticular system.

Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker's syndrome (GSS), and scrapie are transmissible neurodegenerative diseases (1, 5, 16, 26). Attempts to purify the scrapie agent have led to the discovery of a glycoprotein designated prion protein (PrP) (20). The protease-resistant isoform of PrP named PrP^{Sc} or PrP^{CJD} has been implicated as a main component of the infectious agent of scrapie and CJD (20–23). Both the replication of the agent and the accumulation of PrP^{Sc} or PrP^{CJD} occur in the lymphoreticular system, notably in the spleen, long before the involvement of the central nervous system in animals affected by scrapie or CJD (3, 4, 7, 9, 17). The follicular dendritic cell (FDC), a major antigen-presenting cell in lymphoid tissue, is the site of accumulation of PrP^{CJD} in the lymphoreticular system of mice infected by the CJD agent passed serially in mice (11, 17, 19). The accumulation of PrP^{CJD} in FDCs has been observed to occur in the spleen within 30 days after inoculation of the agent whether via the intracerebral (i.c.) or the intraperitoneal (i.p.) route and does not depend on the agent strain (11, 17, 19).

We herein report that the accumulation of PrP^{CJD} in FDCs does not occur in mice inoculated with human CJD material (first-passage mice), whereas it always occurs in mice inoculated with the CJD agent once it has been adapted to the mouse. These phenomena may thus suggest an intense expression of the species barrier in the lymphoreticular system.

We produced first-passage mice by inoculating 20 μl of the brain homogenate from 13 CJD patients (Table 1) to weanling New Zealand White (NZW) mice via the i.c. route as described previously (26). Twelve patients with sporadic cases of CJD (patients 1 to 12) had no mutation in their PrP genes (CJD-wild) except for two heterozygotes with a point mutation at codon 200 (CJD-200) or at codon 232 (CJD-232) (Table 1) (6, 12). All 136 mice that either had died with or without CJD symptoms or had been sacrificed after the appearance of symptoms were autopsied. The brains of all autopsied mice as well as the spleens of 31 autopsied mice were fixed with 10%

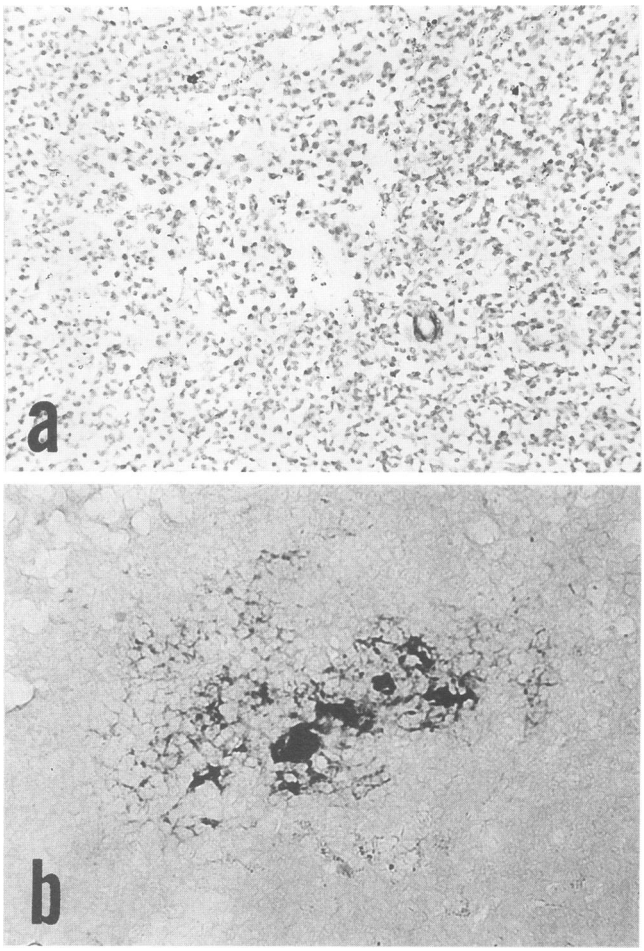

FIG. 1. Immunohistochemical analysis of PrP with anti-PrP-N in the spleens of mice infected with CJD. (a) Spleen of the first-passage mouse infected by transmission from patient 4. No positive immunoreaction is seen. (b) Spleen of the second-passage mouse inoculated with the infectious agent transmitted from patient 4 to a mouse. The

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TABLE 1. Results of transmission from patients with CJD and GSS and PrP immunohistochemistry in the spleens of first-passage mice

Patient	Sex[a]	Age (yr) at onset	Diagnosis	Route of administration	No. of mouse brains examined	No. of mice with positive findings in brain		Mean incubation period ± SD (days)[b]	No. of mouse spleens examined	No. of mice with PrP immunoreaction in FDCs	Period from inoculation to isolation of spleen (days)[c]
						Spongiform changes	PrP immunohistochemistry				
1	F	75	CJD-wild	i.c.	13	10	11	631 ± 79	6	0	651, 663, 665 712, 749, 800
2	F	61	CJD-wild	i.c.	12	5	8	709 ± 134	3	0	616, 670, 833
3	F	63	CJD-wild	i.c.	15	8	13	688 ± 70	3	0	707, 730 (2)
4	M	68	CJD-wild	i.c.	9	6	8	705 ± 77	3	0	394, 600, 637
5	F	47	CJD-200	i.c.	13	6	11	637 ± 81	5	0	606, 643, 658 666, 699
				i.p.	4	0	0		1	0	657
6	M	58	CJD-wild	i.c.	13	6	9	735 ± 61	2	0	693, 833
7	M	59	CJD-wild	i.c.	11	7	11	651 ± 109	2	0	523, 583
8	M	64	CJD-232	i.c.	8	5	8	725 ± 60	2	0	712, 745
9	F	83	CJD-wild	i.c.	14	9	9	643 ± 82	1	0	299
10	F	69	CJD-wild	i.c.	5	5	5	643 ± 93	1	0	665
11	M	74	CJD-wild	i.c.	7	4	5	771 ± 41	1	0	819
12	M	75	CJD-wild	i.c.	8	5	6	704 ± 61	1	0	684
13	M	51	Familial CJD[d]	i.c.	8	6	7	822 ± 77	1	0	742
14	M	61	CJD-wild	i.c.	7	3	5	715 ± 47	0	0	
				i.p.	6	0	0		6	0	210 (4), 260, 359
15	F	68	CJD-wild	i.c.	11	4	7	780 ± 167	0	0	
				i.p.	6	0	0		6	0	210 (4), 267, 368
16	F	55	CJD-wild	i.c.	8	5	7	315 ± 165	0	0	
				i.p.	7	0	0		7	0	210 (4), 347, 365 424
17	M	62	GSS-102	i.c.	16	13	15	253 ± 107	0	0	
				i.p.	4	0	0		4	0	210 (4)

[a] F, female; M, male.
[b] Calculated from the data on mice with PrP immunoreaction in the brain.
[c] Numbers in parentheses indicate numbers of mice whose spleens were isolated after the period.
[d] Analysis of PrP genes has not yet been done.

buffered formalin and embedded in paraffin. Immunohistochemical analysis of PrP was performed on sections of these organs as described previously (17). The peroxidase-antiperoxidase method with hydrolytic autoclaving as pretreatment was used (13); the concentrations of HCl used for the pretreatment were 1.5 or 2.0 mM for the brain and 2.5 or 3.0 mM for the spleen. Two antisera were used as the primary antibodies; one antiserum (anti-PrP-N; 1:200 dilution) was raised against the synthetic polypeptide corresponding to the N-terminal residues of PrP (10), and the other (anti-APC; 1:400 dilution) was raised against amyloid plaque core protein extracted from the brain of a GSS patient (14). Both antibodies reacted with mouse PrP (9, 15, 17). As estimated in a previous study, this immunohistochemical method using these primary antibodies may be 4 to 10 times as sensitive as immunoblotting in detecting PrP^{CJD} in the spleen tissue of the terminal stage of CJD-infected mice (17). In addition, brain sections stained with hematoxylin-eosin were examined histopathologically. Transmission from all of the 13 patients was successful, with the formation of spongiform changes and the accumulation of PrP^{CJD} in the brains of many of the inoculated mice, which were stained equally positive by the two primary antibodies (Table 1) (18). However, PrP^{CJD} was not detected in the spleen of any first-passage mouse irrespective of the inoculum or the primary antibody (Table 1; Fig. 1a).

immunoreaction located in the white pulp has the characteristic features of the FDC, with multiple dendritic processes encasing the surrounding lymphocytes. Magnifications: a, ×200; b, ×280.

To verify how many passages in mice are required for the infectious agent to produce PrP^{CJD} in FDCs, we examined the spleens of NZW mice inoculated i.c. with the 10% homogenate of formalin-fixed brain tissue from the first-passage NZW mouse which had been infected successfully from each of the four CJD-wild cases (patients 1 to 4 in Table 1). Four of the mice inoculated with 20 μl of each material were sacrificed at 120 days after inoculation, approximating the incubation period after the inoculation of mouse-adapted CJD strains (24, 27). PrP^{CJD} which was stained equally positive by both of the primary antibodies was found in FDCs in all of these 16 second-passage mice (Fig. 1b).

It was evident that the accumulation of PrP^{CJD} in FDCs depended on the identity of the species of both the donor and recipient in the transmission. This finding suggested the existence of the species barrier working in the lymphoreticular system against disease transmission. The discrepancy between the findings in the brain and those in the spleen of first-passage mice indicated that the species barrier was expressed more intensely in the lymphoreticular system than in the central nervous system. Further significance of the species barrier in the lymphoreticular system might be suggested by our previous data as follows (11): the severe combined immunodeficiency mouse is the only strain in which PrP^{CJD} did not accumulate in FDCs after inoculation of the mouse-adapted CJD strain Fukuoka-1 (26, 27); in addition, transmission of Fukuoka-1 to the severe combined immunodeficiency mouse via the i.p. route was unsuccessful whereas that via the i.c. route was successful. These data suggest the possibility that the accumulation of PrP^{CJD} in FDCs is an essential step for the i.p.-inoculated CJD

agent to invade the central nervous system. If this is true, then the intense expression of the species barrier in the lymphoreticular system might work against transmission between different species via the i.p. or some other non-i.c. route.

To obtain evidence supporting this theory, we have observed NZW mice inoculated i.p. with 50 μl of the 10% brain homogenate from five CJD or GSS patients (patients 5 and 14 to 17 in Table 1). Patient 17 was a GSS patient with a point mutation at codon 102 of the PrP gene (GSS-102) (8, 25). Transmissions from patients 14 to 17 via the i.c. route had already been successful (Table 1). All four mice inoculated i.p. with the material from patient 5 died free of CJD symptoms near the end of the natural life span (594 to 657 days after inoculation). Of the 12 mice inoculated with the material from each of patients 14 to 17, 4 were sacrificed at 210 days after inoculation, approximating the incubation period after the inoculation of mouse-adapted CJD strains via the i.p. route (19, 27), and none or a few died between 210 and 500 days (Table 1); the other mice all lived asymptomatically for up to 500 days after inoculation. We immunohistochemically examined the brains of all 27 mice which died or were sacrificed as well as the spleens of 24 mice but found no PrP^{CJD} in any of them; this observation was confirmed by the alternate use of the two primary antibodies (Table 1). These data indicated that the species barrier also worked in the lymphoreticular system after i.p. inoculation of the human CJD material. Concurrently, the data documented no transmission from human to mouse via the i.p. route, which agreed with the theory that the barrier works against the transmission between different species via a non-i.c. route.

It might also be worth examining, with our method, the spleens of subjects affected by allied transmissible neurodegenerative diseases, e.g., bovine spongiform encephalopathy (2), whose mode of spread is unknown; such analysis might reveal whether the species barrier is working in the lymphoreticular system of the affected subjects, which might suggest the donor of the infectious agent as well as the mode of spread of the disease.

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