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Evidence for Normal Cell-Mediated Immunity in Scrapie-Infected Mice

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Comparisons between mixed lymphocyte cultures of splenocytes from scrapie-infected and normal mouse brain-inoculated control mice did not reveal any evidence of an impaired cell-mediated immune response in scrapie-infected mice. Likewise, mixed lymphocyte cultures of splenocytes from scrapie-infected and normal mice demonstrated that infected spleen cells had no scrapie-specific antigens on their surfaces. These data suggested that the absence of a detectable scrapie-specific immune response in infected mice was the result of an absence of an exposed scrapie-specific antigen and not due to any direct effect on the immune system.

Mixed lymphocyte culture reactions are uniquely able to detect cell surface differences, even when unimmunized cells are used. A large fraction of the cells (1 to 3 percent) are capable of responding to such cell surface differences as the histocompatibility antigens (7). Because of the sensitivity of mixed lymphocyte reactions, they are useful for measuring not only antigenic structures of cell surfaces but also functional capacities of cellular immune systems.

We tested the ability of splenic T lymphocytes from scrapie-infected mice to respond in mixed lymphocyte cultures with both infected and uninfected spleen cells, and we examined the possibility that infected murine spleen cells may have a scrapie-specific antigen on their surfaces which can be detected in mixed lymphocyte cultures with responder cells from normal mice. These studies demonstrated that there was no impairment of T-cell function in infected mice and that there was no exposed cell surface scrapie-specific antigen detectable in mixed lymphocyte cultures.

MATERIALS AND METHODS

Mice. BALB/c and C57BL/6 mice were obtained from the Lionel Strong Foundation, Del Mar, Calif., and were used to establish the breeding colony which supplied the mice for these experiments. C3H/HeJ female mice were obtained from the Jackson Laboratories, Bar Harbor, Maine.

Scrapie agent. We used the C506 strain of American scrapie in the fourth mouse passage, which was originally isolated directly in mice from a naturally affected Suffolk ewe (6). The mouse brain suspension which we used as an inoculum titrated 3.3×10^9 50 percent lethal doses per ml and was free of all murine viral agents cytopathic for primary mouse embryo and BHK-21 C13 cell cultures.

Infection. Each mouse was infected by intracerebral inoculation of 0.03 ml of a 1:10 dilution of mouse brain homogenate in phosphate-buffered saline (pH 7.4). Each animal received approximately 1.1×10^7 50 percent lethal doses. Control mice were inoculated similarly with an identical preparation of normal mouse brain homogenate. As a control for the infectivity of the scrapie agent, mice from each of the scrapie-inoculated groups were held until disease developed.

Mixed lymphocyte culture. Mouse spleen cells were used as both the responding cells and the stimulating cells. Stimulating cells were preincubated with mitomycin C (25 μg/ml) at 37°C for 30 min; this preincubation was followed by three washes in complete Eagle minimum essential medium. Either 2.5×10^5 or 5×10^5 stimulating cells were mixed with an equal number of responding cells in 200 μl of Eagle minimal essential medium containing nonessential amino acids, 5 percent fetal calf serum (Irvine Scientific, Santa Ana, Calif.), 5×10^{-5} M 2-mercaptoethanol, 1 mM glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml in a well of a Microtest II culture plate (Falcon Plastics, Oxnard, Calif.). The cultures were maintained at 37°C in an atmosphere containing 5 percent CO_2 in air in a humidified chamber. After 96 h the cultures were labeled radioactively for 6 h with 0.25 μCi of [^{3}H]thymidine (New England Nuclear Corp., Boston, Mass.) and harvested with a MASH II harvester (Microbiological Associates, Bethesda, Md.). The cell precipitates were collected on glass fiber filters, which were dried thoroughly and counted in a toluene-based liquid scintillation counting fluid.

Mitogenesis of spleen cell cultures. Single-cell suspensions of splenocytes were prepared as described above and were cultured at a concentration of 5×10^5 cells per 200 μl. The mitogens studied were concana-

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valin A and two forms of *Escherichia coli* lipopolysaccharide (LPS); one LPS was a trichloroacetic acid extract of *E. coli* O55:B5 and was obtained from Difco Laboratories, Detroit, Mich., and the other LPS was a more purified preparation, which was extracted from *E. coli* K235 and was provided by J. Watson. Mitogenesis was measured by [^{3}H]thymidine uptake as described above.

RESULTS

The spleen weights of infected and control (normal mouse brain-inoculated) mice were determined carefully throughout this study (Table 1). The spleens were removed, carefully trimmed of peritoneal fat, and weighed individually. Each mouse was weighed at the time of sacrifice, and this figure was used to determine the relative spleen weight (spleen weight/mouse weight). No obvious splenomegaly was present in the infected BALB/c and C57BL/6 mice throughout the infection. Mild splenomegaly was observed in the C3H/HeJ mice on days 42 and 49.

TABLE 1. *Spleen weights of scrapie-infected and control mice at different times after infection*

Mice	Time after inoculation (days)	Relative spleen wt[a]	
		Scrapie infected	Control
C3H/HeJ	14	4.1 ± 0.1[b]	4.7 ± 0.1
	21	4.8 ± 0.1	4.8 ± 0.1
	28	4.8 ± 0.1	4.7 ± 0.1
	35	4.6 ± 0.1	4.6 ± 0.1
	42	6.1 ± 0.1	5.3 ± 0.1
	49	5.9 ± 0.2	4.9 ± 0.1
	63	4.6 ± 0.1	4.2 ± 0.2
	70	4.5 ± 0.1	4.4 ± 0.2
BALB/c	12	5.4 ± 0.1	5.5 ± 0.2
	15	5.6 ± 0.1	5.4 ± 0.1
	16	5.5 ± 0.2	5.2 ± 0.1
	17	5.7 ± 0.1	5.7 ± 0.1
	18	5.1 ± 0.1	5.7 ± 0.2
	19	6.0 ± 0.2	5.5 ± 0.2
	20	5.8 ± 0.3	5.2 ± 0.1
	30	6.0 ± 0.2	5.7 ± 0.3
	35	6.1 ± 0.1	5.9 ± 0.1
	42	5.9 ± 0.4	5.7 ± 0.2
	49	6.0 ± 0.3	5.6 ± 0.3
	63	5.8 ± 0.2	5.6 ± 0.4
	70	5.6 ± 0.4	5.2 ± 0.3
C57BL/6	12	5.3 ± 0.2	5.2 ± 0.3
	17	5.6 ± 0.1	5.4 ± 0.2
	20	4.9 ± 0.2	4.8 ± 0.1
	30	4.8 ± 0.2	5.2 ± 0.3
	35	4.9 ± 0.1	5.0 ± 0.2
	42	5.7 ± 0.3	4.8 ± 0.1
	49	5.0 ± 0.2	4.6 ± 0.3
	63	5.4 ± 0.3	4.8 ± 0.1
	70	5.0 ± 0.2	4.9 ± 0.2

[a] Data are averages for four or more mice of the weight of the spleen divided by the weight of the mouse (11).

[b] Mean ± standard deviation expressed in milligrams per gram.

Ability of scrapie-infected spleen cells to react in mixed lymphocyte cultures. An in vitro stimulation system was used to test the ability of splenocytes from scrapie-infected mice to recognize and respond to foreign cell surface antigens by using the H-2 complex as an antigen. Splenic lymphocytes from scrapie-infected and normal mouse brain-inoculated mice were cultured together with mitomycin C-treated cells from the same strain or from an H-2 incompatible strain (Table 2). The responses of the spleen cells from scrapie-infected animals were examined through the first 10 weeks of the infection, and both BALB/c and C57BL/6 mice were studied. The results clearly indicated no measurable difference between infected and normal mice. The kinetics of the anti-H-2 response were also examined by labeling at 24, 36, 48, 72, and 96 h of culture. There was no detectable difference between the scrapie-infected and control animals. Spleen cell preparations were also normal in their responses to the B-cell mitogen LPS and the T-cell mitogen concanavalin A.

Mixed lymphocyte cultures of normal spleen cells and scrapie-infected stimulator cells. There is very strong evidence that the scrapie agent is associated almost exclusively with the cell membrane; therefore, if a scrapie antigen of any type is present in infected cells, the cell surface should be the best place to look. To examine cell surfaces for any new antigenic structures, a series of mixed lymphocyte reactions were performed by using responder cells from normal uninoculated 8- to 10-week-old mice and stimulator cells from the spleens of mice at varying times up to 10 weeks postinfection. The spleens should have reached maximum infectivity during this period (Table 3).

Three different strains of mice were used as sources of stimulator spleen cells, whereas only BALB/c and C57BL/6 spleen cells were used as responders. In each case, the stimulator cells were treated with mitomycin C before use. We found that there was no measurable stimulation of responder spleen cell deoxyribonucleic acid synthesis that could be attributed to a scrapie antigen. Although the responder cells were stimulated strongly by the H-2 antigens, there was no evidence of any other type of cell surface difference.

Mitogenic stimulation of C3H/HeJ spleen cells with concanavalin A and LPS. Spleen cell cultures from C3H/HeJ mice were examined for their responses to the mitogens LPS and concanavalin A. The mice were examined at weekly intervals between 14 and 70 days after

TABLE 2. *Mixed lymphocyte response of splenic lymphocytes from scrapie-infected and control mice at different times after infection*

Days after inoculation	Inoculum	Stimulation index with mixtures of splenocytes from:[a]			
		BALB/c mice and mitomycin C-treated BALB/c mice	BALB/c mice and mitomycin C-treated C57BL/6 mice	C57BL/6 mice and mitomycin C-treated C57BL/6 mice	C57BL/6 mice and mitomycin C-treated BALB/c mice
12	Control[b]	1.00 ± 0.09	7.56 ± 0.48	1.00 ± 0.08	7.01 ± 0.37
	Scrapie	0.95 ± 0.25	7.38 ± 0.62	0.99 ± 0.07	7.40 ± 0.51
18	Control	1.00 ± 0.07	5.33 ± 0.42	1.00 ± 0.06	4.35 ± 0.24
	Scrapie	0.94 ± 0.06	6.05 ± 0.44	0.76 ± 0.06	4.62 ± 0.22
21	Control	1.00 ± 0.07	7.21 ± 0.33	1.00 ± 0.08	6.74 ± 0.36
	Scrapie	0.96 ± 0.08	7.77 ± 0.52	0.92 ± 0.07	6.60 ± 0.31
28	Control	1.00 ± 0.07	5.69 ± 0.32	1.00 ± 0.09	5.69 ± 0.36
	Scrapie	0.93 ± 0.08	5.82 ± 0.37	0.99 ± 0.07	5.44 ± 0.41
30	Control	1.00 ± 0.07	6.61 ± 0.31	1.00 ± 0.08	6.85 ± 0.37
	Scrapie	0.91 ± 0.08	6.78 ± 0.40	0.93 ± 0.08	6.77 ± 0.40
35	Control	1.00 ± 0.06	8.38 ± 0.43	1.00 ± 0.08	6.19 ± 0.40
	Scrapie	0.85 ± 0.05	7.81 ± 0.46	0.82 ± 0.07	6.29 ± 0.32
42	Control	1.00 ± 0.08	8.41 ± 0.67	1.00 ± 0.08	7.66 ± 0.54
	Scrapie	1.20 ± 0.15	7.71 ± 0.70	1.12 ± 0.10	7.43 ± 0.53
49	Control	1.00 ± 0.09	7.11 ± 0.45	1.00 ± 0.08	8.35 ± 0.39
	Scrapie	1.07 ± 0.07	7.29 ± 0.51	0.89 ± 0.07	7.68 ± 0.37

[a] Mixtures contained equal numbers of mitomycin C-treated and untreated lymphocytes. The cultures were labeled for 6 h with 0.25 μCi of [^{3}H]thymidine after 96 h in culture. Each stimulation index was determined by dividing the counts per minute of the stimulated culture by the counts per minute of the comparable control. Each value is the mean ± standard deviation of triplicate wells.

[b] Control mice were inoculated with a suspension of normal mouse brain.

TABLE 3. *Mixed lymphocyte responses of normal BALB/c splenic lymphocytes with splenic lymphocytes from scrapie-infected and control mice*

Days after inoculation	Inoculum	Stimulation index with the following stimulator cell types:[a]		
		BALB/c	C3H/HeJ	C57BL/6
12	Control[b]	1.00 ± 0.08	7.40 ± 0.37	7.97 ± 0.33
	Scrapie	1.02 ± 0.07	7.13 ± 0.46	8.14 ± 0.36
14	Control	1.00 ± 0.11	6.45 ± 0.34	7.12 ± 0.25
	Scrapie	0.94 ± 0.07	6.31 ± 0.28	6.15 ± 0.34
18	Control	1.00 ± 0.08	7.87 ± 0.28	7.64 ± 0.27
	Scrapie	1.06 ± 0.08	7.00 ± 0.42	8.16 ± 0.31
21	Control	1.00 ± 0.08	8.01 ± 0.68	7.68 ± 0.66
	Scrapie	1.01 ± 0.08	7.10 ± 0.60	7.79 ± 0.46
28	Control	1.00 ± 0.17	7.96 ± 0.66	8.88 ± 0.59
	Scrapie	1.04 ± 0.10	7.29 ± 0.68	8.37 ± 0.36
30	Control	1.00 ± 0.09	10.62 ± 0.77	10.30 ± 0.75
	Scrapie	0.85 ± 0.15	9.01 ± 0.76	9.44 ± 0.59
35	Control	1.00 ± 0.25	9.84 ± 0.71	9.20 ± 0.83
	Scrapie	0.95 ± 0.08	9.01 ± 0.62	10.33 ± 0.76
42	Control	1.00 ± 0.19	10.30 ± 1.11	11.14 ± 1.12
	Scrapie	1.05 ± 0.31	9.37 ± 0.95	10.24 ± 0.84
49	Control	1.00 ± 0.15	8.50 ± 0.86	9.25 ± 1.43
	Scrapie	0.97 ± 0.25	8.83 ± 0.87	9.51 ± 1.25
63	Control	1.00 ± 0.18	9.81 ± 0.83	11.57 ± 1.64
	Scrapie	0.98 ± 0.19	9.92 ± 1.23	11.58 ± 1.74
70	Control	1.00 ± 0.24	9.31 ± 0.85	9.90 ± 0.85
	Scrapie	0.97 ± 0.16	8.83 ± 0.79	9.13 ± 0.76

[a] The cultures were labeled for 6 h with 0.25 μCi of [^{3}H]thymidine after 96 h in culture. The stimulation index was determined by dividing the counts per minute in each stimulated culture by the counts per minute in the comparable control. Each value is the mean ± standard deviation of quadruplicate wells.

[b] Control animals were inoculated with a suspension of normal mouse brain.

infection. Although mild splenomegaly occurred on days 42 and 49, these changes were not reflected in changes in the mitogenic responses of these spleens (Table 4). Although there was wide variation from mouse to mouse, there was no consistent pattern of depression of the LPS mitogenic response. In those instances where individual mice were compared as a result of their spleen weights, there was no correlation between an enlarged spleen and a depression of the mitogenic response to LPS.

DISCUSSION

Although scrapie has been recognized as a transmissible infectious disease for many years, there are many features of this disease which distinguish it from diseases caused by conventional infectious agents. One of the most striking features of scrapie is the lack of any demonstrable scrapie-specific immune response in infected hosts. This feature of the disease is apparent not only in the natural hosts, sheep and goats, but also in a variety of experimental animals to which it has been adapted. Regardless of the host species, the pathologic lesions are similar and appear to be confined to the central nervous system despite the wide dissemination of the agent throughout the hosts (9). Previous work has established the lack of humoral immunity in scrapie-infected mice (12) and the apparent normal mitogenic response of splenic lymphocytes to both T- and B-cell mitogens (4).

The pathogenesis of scrapie in mice involves the spleen at an early stage (2), and the maximum spleen titers appear before the development of any apparent histopathological changes in the brain. The rapid involvement of the spleen and the lack of any form of immunity even in animals with apparently functional immune systems with regard to other antigens (1, 3) have raised questions regarding the interactions of the scrapie agent with thymus-derived cells, which may play a helper role in humoral immunity or in cell-mediated immunity. McFarlin et al. (10) showed that functional T lymphocytes are not necessary for infection and that thymectomized mice have incubation periods identical to those of normal mice. However, the experiments of these authors did not address the functional capabilities of the thymus-derived cells from infected animals.

In this study, we examined a large number of infected mice for several parameters of cell-mediated immunity and lymphocyte function. The splenomegaly reported by others (5) was absent in these mice, with the possible exception of a transient change between 42 and 49 days postinfection in C3H/HeJ mice. Likewise, the alteration of the LPS mitogenic response in the C3H/HeJ mice reported by Garfin et al. (5) was not detected in this study. The reason for these differences is not clear, but there are two possible explanations. (i) The two studies were done with different strains of the scrapie agent, one from England, which was passed repeatedly

TABLE 4. *Mitogenic stimulation by LPS and concanavalin A of C3H/HeJ splenocytes from scrapie-infected and control mice*

Days after inoculation	Inoculum	Stimulation index with:[a]				
		LPS-W (50 μg/ml)[b]	LPS-D (100 μg/ml)[b]	LPS-D (50 μg/ml)	LPS-D (10 μg/ml)	Concanavalin A (2.5 μg/ml)
21	Scrapie	1.03 ± 0.1	7.1 ± 1.1	3.9 ± 0.4	4.6 ± 0.9	20.5 ± 5.4
	Control[c]	1.3 ± 0.3	6.3 ± 3.4	3.9 ± 1.5	4.8 ± 1.5	20.7 ± 6.6
28	Scrapie	1.1 ± 0.1	6.4 ± 1.1	3.5 ± 0.3	3.7 ± 1.5	18.2 ± 4.8
	Control	0.9 ± 0.2	5.9 ± 1.3	4.1 ± 1.0	3.8 ± 1.3	19.7 ± 2.2
35	Scrapie	1.0 ± 0.3	4.2 ± 0.8	2.7 ± 0.7	3.7 ± 0.6	19.5 ± 5.7
	Control	0.9 ± 0.1	4.2*1.2	2.4 ± 0.4	3.4 ± 0.6	19.0 ± 3.7
42	Scrapie	1.1 ± 0.1	6.9 ± 2.0	3.2 ± 0.8	4.2 ± 1.0	11.9 ± 3.6
	Control	1.0 ± 0.2	4.2 ± 0.8	2.2 ± 0.4	3.6 ± 1.4	12.0 ± 3.9
49	Scrapie	1.1 ± 0.2	4.4 ± 2.8	2.4 ± 1.1	2.9 ± 1.5	12.4 ± 1.0
	Control	0.9 ± 0.3	5.6 ± 1.7	2.9 ± 0.6	3.2 ± 1.1	15.5 ± 3.5
63	Scrapie	0.7 ± 0.1	2.5 ± 0.4	2.5 ± 0.3	3.8 ± 0.5	13.4 ± 2.8
	Control	0.8 ± 0.2	2.7 ± 0.7	3.4 ± 0.7	4.0 ± 0.8	13.3 ± 2.2
70	Scrapie	0.8 ± 0.2	2.6 ± 0.1	1.8 ± 0.6	3.3 ± 0.5	4.9 ± 1.2
	Control	0.9 ± 0.2	2.0 ± 0.8	1.9 ± 0.4	2.8 ± 0.7	6.8 ± 2.0

[a] The stimulation indexes were determined by dividing the counts per minute of stimulated cultures by the counts per minute of the comparable unstimulated cultures. Each determination was the average of triplicate wells and four or more mice per determination.

[b] LPS-W was extracted from *E. coli* K235 and was obtained from James Watson; LPS-D was extracted from *E. coli* O55:B5 and was purchased from Difco Laboratories.

[c] Control mice were inoculated with a suspension of normal mouse brain.

through goats before its transmission to mice, and was later shown to be composed of two different strains (8) and the other from the United States, which was isolated directly in mice. (ii) There may have been a second agent in the stock pool used by Garfin et al. (5), which led to spleen alterations. A direct comparison in a single laboratory of animals infected by the two strains may be required to resolve these conflicting results.

Lack of immunity in scrapie could stem from several factors. Previous workers have shown that antibody production in scrapie-infected animals is normal to all of the antigens which have been tested (1, 3); therefore, the lack of a humoral response is due to either the absence of a scrapie antigen, the loss of specific T or B lymphocytes, or the scrapie antigen not being recognized as foreign due to the nature of its association with the cell membrane.

There have been no previous examinations of the functional capacity of T lymphocytes from scrapie-infected animals to mount a cellular immune response. Although the T-helper functions had to be intact, as shown by the functional humoral response, no measurement of any other T-cell population has been made. There are no obvious impairments of the immune systems of scrapie-infected mice. Spleen cells from infected mice are as active in mixed lymphocyte culture reactions as spleen cells from control mice.

The absence of immune stimulation in mixed lymphocyte cultures of uninfected spleen cells and scrapie-infected spleen cells suggests that the lack of immune reactivity in scrapie-infected cells is due to the absence of an exposed antigen. These experiments were done during the period of maximum infectivity of the spleens and if a cell surface antigen existed, it would have been present during that time. Although the exact molecular form of the scrapie agent is unknown, the maximum infectivity resides with the cell membrane (6). The absence of immune reactivity and the inability of infected cells to stimulate in vitro blast transformation suggest that whatever macromolecular structures are associated with, the scrapie virus are most likely buried in the core of the membrane or, alternatively, are nonantigenic. Any further understanding of these observations must await the final molecular characterization of the scrapie virus.

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