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Major histocompatibility complex genes have an increased brain expression after scrapie infection

(class I and class II genes/β_2-microglobulin/invariant chain/immunohistochemistry)

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ABSTRACT **We have examined the expression of the major histocompatibility complex (MHC) antigens and related genes in scrapie-infected hamster brain. Both the class I and the class II MHC genes as well as the class II-associated invariant chain were found to have an increased brain expression after scrapie infection. The increased expression of the class I complex was immunohistochemically localized primarily to neurons, though some astrocytes contained much smaller amounts of the class I complex. While there is no detectable immune response to scrapie infection, the possibility that increased MHC expression affords some defense against the scrapie agent is discussed.**

Scrapie is a transmissible neurodegenerative disease of the sheep and goat and is an animal model for human slow virus diseases, including kuru and Creutzfeldt–Jakob disease (1). The agent is resistant to many virucidal and nucleic acid inactivating agents, and Prusiner (2) has postulated that the agent lacks a nucleic acid genome in his prion hypothesis. No typical nucleic acid genome has been identified in highly purified preparations of the agent (3–5), although a segment of mitochondrial DNA has been isolated from this material (6). Abnormal fibrils were first demonstrated in scrapie-infected mouse brain (7) and were subsequently shown to be highly specific for slow viral infections in general (8). The primary macromolecule found in these preparations was a 27- to 30-kDa glycoprotein (9, 10) which, although host-encoded, has been shown to have altered properties after scrapie infection (3–5).

β_2-Microglobulin has an increased expression in scrapie-infected hamster brain (11). β_2-Microglobulin is associated with the class I major histocompatibility complex (MHC) gene product to form the class I complex, which is expressed in most organs of the body and presents antigen to T cells in the class I-restricted immune response (12). While this complex is not detectable in normal brain parenchyma, it is expressed on both neurons and glial cells after viral infection (13, 14) or experimental manipulation (15).

In light of the finding that the brain expression of β_2-microglobulin is increased in scrapie, we have examined the expression of class I and class II MHC genes and those of associated proteins in this disease. The implication of our findings for understanding the host response to scrapie infection is discussed.

METHODS AND MATERIALS

Tissue. Late-clinical-stage golden Syrian hamsters infected by intracerebral injection of the 263K strain of the agent (16) were used in this study. The RNA blot results reported here were obtained using the same infected and mock-infected control brain RNAs that were used previously (17). These results were confirmed using tissue from two independent sources, from R. Race and B. Chesebro (Rocky Mountain Laboratories, Hamilton, MT) and from J. Aiken and R. Marsh (University of Wisconsin, Madison); these samples were also used for immunohistochemistry. Hamsters were treated with cuprizone or triethyltin bromide (Aldrich) as described (8). Cuprizone (0.75% of diet on a weight basis) was given for 8 weeks, and triethyltin bromide (30 mg/ml in drinking water) was administered for 2 weeks, after which the animals were sacrificed by CO_2 narcosis.

RNA Blot Analysis. RNA was isolated, electrophoresed, and blotted as described (17). Blots were hybridized with recombinant and synthetic probes generated from agarose gel-purified gene fragments (18). The probe for the highly conserved MHC class I $\alpha 3$ region was obtained by using synthetic oligonucleotides representing the opposite ends of the $\alpha 3$ exon as PCR primers, with murine genomic DNA as template (11). The MHC class I oligonucleotides represented residues 402–425 and 628–608 of the murine sequence (19). A single band of appropriate length was obtained and the identity of the amplified gene segment was established by DNA sequence analysis (17). Recombinants for the murine invariant chain (20) and the rat class II α chain (21) were obtained from the cited investigators.

Immunohistochemistry. Late-stage scrapie-infected hamster brain was cut into 2-mm sagittal sections and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS: 20 mM sodium phosphate/100 mM NaCl, pH 7.4) for 2 hr. The tissue was then dehydrated and embedded in paraffin, from which 10-μm sections were cut and mounted on slides. Sections were incubated first for 20 min in 5% bovine serum albumin in PBS, then with the Vector Laboratories avidin–biotin blocking kit as described by the manufacturer. Sections were then incubated with a rabbit polyclonal antibody against rat β_2-microglobulin (Serotec), which has been reported to crossreact with the hamster β_2-microglobulin (22). The primary antibody incubation and its detection with the Vector Laboratories ABC Elite peroxidase kit were performed as recommended by the manufacturer. Reactive astrocytes were subsequently identified by incubating the previously stained section with rabbit antibody against human glial fibrillary acidic protein (Incstar, Stillwater, MN), detected as above. Nuclear morphology was established by subsequent staining with Harris hematoxylin (Anatech, Battle Creek, MI).

RESULTS

The brain expression of the class I gene was examined by using a probe derived from the $\alpha 3$ region of this gene. RNA blot analysis showed a significantly increased expression of this gene in scrapie-infected hamster brain compared with

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Abbreviation: MHC, major histocompatibility complex.

controls (Fig. 1 *Top*), similar to the situation found previously for β_2-microglobulin (11).

The class II α-chain probe yielded a faint signal with the scrapie RNAs, but no signal was detectable in the control RNAs (Fig. 1 *Middle*). The expression of the class II-associated invariant chain was found to be increased after scrapie infection to a degree similar to that of the class I gene (Fig. 1 *Bottom*).

The systemic expression of these genes was examined in scrapie-infected and control animals. The spleen has the highest scrapie titers of extraneural tissues after intracerebral innoculation, though these titers are much lower than those found in the brain (26, 27). There was no significant alteration of the expression of these genes in spleen after scrapie infection (Fig. 2).

The neurotoxins triethyltin and cuprizone cause a spongiform brain degeneration reminiscent of scrapie (8). Neither of these toxins affected the brain expression of any of these genes (Fig. 3).

The distribution of the class I complex in scrapie-infected hamster brain was examined immunohistochemically with antibody against β_2-microglobulin. Cellular staining was most prominent in the middle layers of the cerebral cortex (Fig. 4*A*); their large polygonal shape suggested these cells to be neurons. Subsequent staining with antibody against glial fibrillary acidic protein identified two reactive astrocytes with their stellate cytoplasm (Fig. 4*B*). On close examination of Fig. 4*A*, it can be seen that one of these astrocytes reacted weakly with antibody against β_2-microglobulin while the other was unreactive; in other fields the majority of astrocytes were unreactive. Staining the section with hematoxylin revealed the large, circular nuclei of the cells strongly stained with antibody against β_2-microglobulin, confirming their identification as neurons (Fig. 4*C*). There was minimal parenchymal staining in control brain, though there was staining of blood vessels (Fig. 4 *D* and *E*), consistent with the findings of other investigators (15, 28). The difference in neuronal nuclear morphology in Fig. 4 *C* and *E* resulted from the previous peroxidase reaction of neurons in Fig. 4*C*.

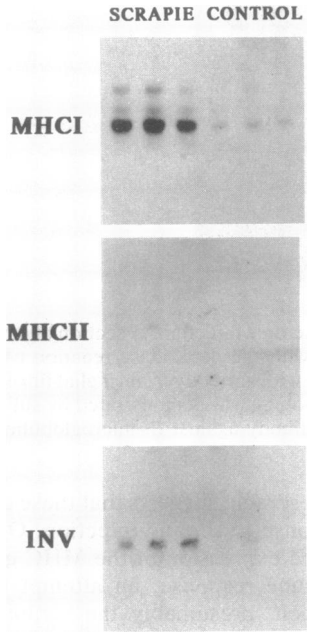

FIG. 1. Brain expression of MHC-related genes after scrapie infection. RNA blots with 10 μg of total brain RNA from three scrapie-infected hamsters and three controls were hybridized with probes for MHC class I and class II genes (2-day autoradiographic exposure) and the invariant (INV) chain (3-day exposure). The lengths of the primary transcripts identified by these probes were 1.5, 1.25, and 1.5 kilobases, respectively, approximating the lengths determined by sequence analysis (23–25).

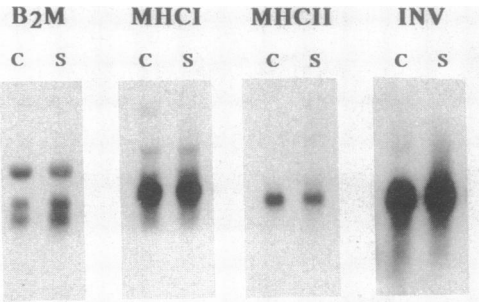

FIG. 2. Systemic expression of MHC-related genes after scrapie infection. RNA blots with 10 μg of spleen RNA from a scrapie-infected hamster (lanes S) and a control (lanes C) were hybridized with probes for β_2-microglobulin (β_2M), MHC class I and class II genes, and the invariant (INV) chain. Autoradiograms were exposed for 16 hr.

DISCUSSION

The brain expression of the class I and class II genes, as well as the class II-associated invariant chain, was found to be increased after scrapie infection. No increased expression of these genes was found in the spleen following scrapie infection, suggesting that this response is restricted to the brain. The brain expression of these genes was not affected by either triethyltin or cuprizone, two neurotoxins that cause a spongiform change in the brain (8), which suggests that there is some specificity of this pattern of altered gene expression for scrapie.

The class I complex is involved in the binding and presentation of cytoplasmic antigens (typically viral antigens) to the immune system, primarily to cytotoxic T cells, whereas the class II complex, an α-chain/β-chain dimer, is responsible for the binding and presentation of circulating antigens to helper T cells (15, 29). While the increased expression of the class

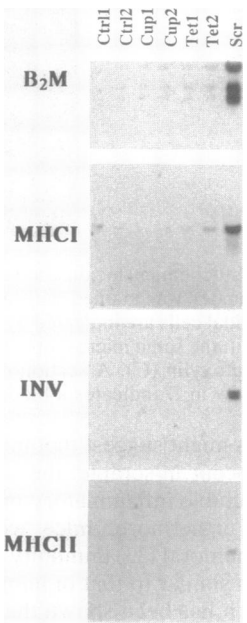

FIG. 3. Effect of neurotoxins on brain gene expression. Brain RNA was isolated from two hamsters treated with cuprizone (Cup) and two hamsters treated with triethyltin (Tet) (8). RNA blot analysis with probes as in Fig. 2 was used to examine the changes in brain gene expression brought on by these toxins compared with two control hamsters (Ctrl) and one scrapie-infected hamster (Scr).

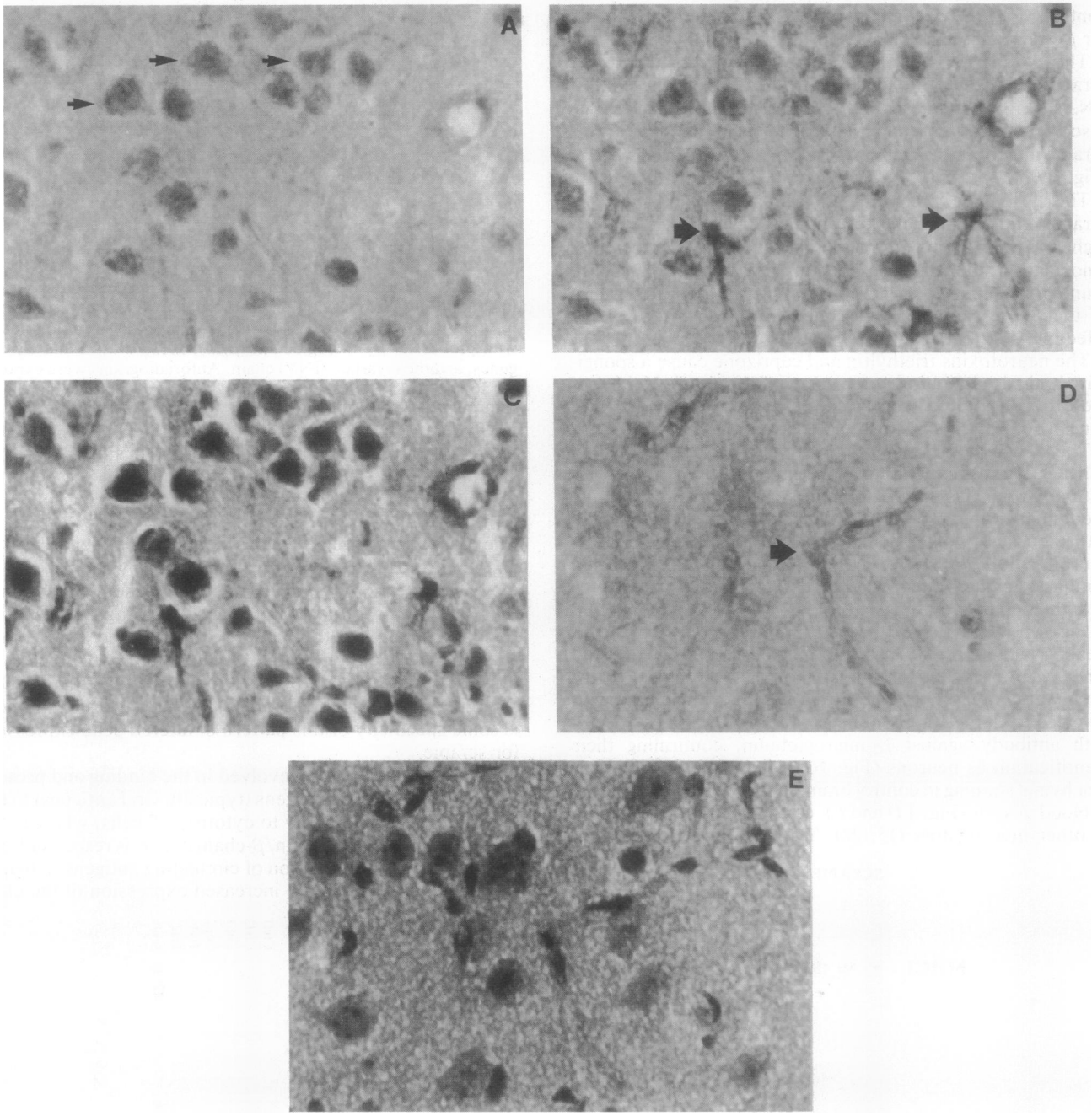

FIG. 4. Immunohistochemical localization of class I complex expression in the brain after scrapie infection. A section of cerebral cortex from a scrapie-infected hamster was stained with antibody against β_2-microglobulin, visualized with the peroxidase reaction (*A*). Representative darkly staining pyramidal cells are indicated by arrows. The section was then stained similarly with antibody against glial fibrillary acidic protein to identify astrocytes in the same microscopic field, indicated with arrows (*B*). Nuclear morphology was demonstrated by subsequently staining the section with hematoxylin (*C*). A section of control hamster cortex was stained with antibody against β_2-microglobulin (*D*), followed by hematoxylin (*E*). Arrow in *D* indicates a blood vessel. (×570.)

I and II MHC genes might suggest that the early events of an immune response occur in scrapie, the slow viral infections do not elicit a detectable inflammatory or immune response in the host (1). Furthermore, mice with deficiencies of cellular (30–32) or humoral (33) immunity have a course after slow virus infection similar to that of immunologically intact animals. However, it has been shown that macrophages can clear scrapie preparations of infectivity (34), which suggests that there is some host defense against the infection.

The increased brain expression of the class I gene we have found in scrapie sheds light on the previous finding of increased expression of β_2-microglobulin in this condition (11). The coordinate increased expression of the class I and II MHC genes in scrapie suggests that these genes play a role in the host response to scrapie infection. One possibility is that the increased expression of the MHC genes represents an aborted immune response, an attempt by the brain to present an antigen, presumably the scrapie agent, to the immune system, against which it is not demonstrably reactive. Despite the absence of a detectable immune response in scrapie, the increased expression of the class I and class II genes may represent a defensive response against the agent; with the increased antigen-binding capacity provided by this increase in MHC expression, the agent could be sequestered and its replication inhibited. It is of interest that the primary site of increased class I complex accumulation in the scrapie-

infected brain is the neuron, the destruction of which is a hallmark of the disease.

One finding which suggests that increased MHC expression is an important part of the host response to scrapie infection is that one of the two genetic loci found to determine the scrapie incubation time (35) maps to the D subregion of the murine H-2 complex (33, 36), which among many other loci, contains the class I gene (37). In sheep, scrapie resistance has also been reported to be linked to the MHC (38), though this finding has been disputed (39). This locus in the mouse was considered to be novel and was called PID-1 (33). Our finding that class I genes have an increased expression after scrapie infection suggests the possibility that the PID-1 locus represents a class I gene. Animals with relatively resistant MHC haplotypes might have a class I complex that more effectively binds the scrapie agent and slows the infection, whereas the more sensitive MHC haplotypes might code for class I molecules that bind the agent weakly. While it has been shown that class I molecules bind processed antigens (40) and the antigen binding site of the class I molecule can accommodate a 20-amino acid peptide, one end of the antigen-binding cleft is open and a larger antigen could be bound (41). A similar model has been proposed for the effectiveness of different MHC haplotypes in the classical immune response against lymphocytic choriomeningitis virus, where, as with the PID-1 locus in scrapie (33), the resistant MHC haplotype is dominant over the sensitive one (28).

Alternatively, since both the cellular isoform of the scrapie agent protein (42, 43) and the MHC antigens are expressed on the cell surface, there might be a cooperative interaction between these proteins during scrapie infection that results in increased MHC expression. The finding that the cellular isoform of the scrapie agent protein expressed on lymphocytes participates in mitogen-induced activation (42) suggests that such interactions are possible.

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