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# Multiple Sclerosis: Altered Expression of 70- and 27-kDa Heat Shock Proteins in Lesions and Myelin

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**Abstract.** Recent studies have implicated heat shock proteins (HSP) in the pathogenesis of the multiple sclerosis (MS) lesion. Expression of the 73 kDa constitutive HSP (HSC70), the 72 kDa stress-inducible HSP (HSP70), and the 27 kDa small HSP (HSP27) was analyzed in white matter and myelin from central nervous system (CNS) tissue of MS and normal subjects using a combination of immunocytochemistry and quantitative immunoblotting. Plaques of all types were sharply defined by reduced immunostaining for HSC70, and shown by immunoblotting to contain 30 to 50% less HSC70 than surrounding white matter or normal tissue. In contrast, HSP27 was markedly enhanced 2.5- to 4-fold in plaque regions, especially in fibrous astrocytes and in hyperplastic interfascicular oligodendrocytes at the lesion edge. HSP70 was less abundant than HSC70, and no significant differences in HSP70 levels were noted between MS and normal white matter. Myelin isolated from active plaques contained 3- to 4-fold more HSC70 than normal myelin. Pronounced expression of HSP70 and HSP27 was also found in MS myelin, although neither protein was detected in normal myelin. Thus, white matter undergoing immune-mediated destruction in MS was associated with altered distribution and expression of HSC70 and HSP27. These changes may initially serve to protect myelin from further destruction and facilitate repair; however, enhanced expression of HSC70, HSP70, and HSP27 in myelin may subsequently present as additional immune targets involved in the progression of disease.

## INTRODUCTION

When exposed to elevated temperatures or other adverse conditions, cells from all organisms respond by upregulating the expression of a class of proteins known as heat shock proteins (HSP) that are thought to function in a universal cellular defense mechanism (1). Although first identified as being induced under conditions of stress, most HSP exist constitutively and have been shown to be important to the normal functioning of a cell. The most widely studied HSP are members of the 70 kDa family, which includes the inducible 72 kDa HSP70 and the constitutive 73 kDa HSC70. These 2 proteins are generally localized in the cytosol (however, they can also be found in the nucleus, particularly following stress), exhibit extensive sequence homology (>90%), interact with one another, and perform similar functions (2). It is unclear why both of these proteins are necessary. However, our findings and those of others suggest that different transcriptional and translational control mechanisms regulate HSP70 and HSC70 (3–5).

The role(s) of HSP in multiple sclerosis (MS), an inflammatory demyelinating disease of the human central nervous system (CNS), has recently increased in prominence. Interest in HSP in MS stems mostly from the high immunogenicity and evolutionary conservation of HSP. These properties have suggested that HSP can act as potential autoantigens and trigger, amplify, and/or modify an autoimmune response. Several lines of evidence support this hypothesis: (a) increased expression of HSP has been noted at the cartilage-pannus junction in rheumatoid joints and in β-cells of the pancreas in autoimmune forms of arthritis and diabetes, respectively; (b) HSP-reactive T cells have been detected in these autoimmune diseases; (c) in adjuvant arthritis, an animal model, disease can be adoptively-transferred to naive animals by HSP-reactive T cell lines; and (d) both adjuvant arthritis and autoimmune mouse diabetes can be blocked by immunization with HSP antigen using a regimen that results in the induction of tolerance rather than sensitization (6–11). Several laboratories have shown an elevated T cell response to the 60- and 70-kDa HSP families in patients with MS (12–15). In addition, van Noort and colleagues (16) detected αB crystallin (a 23 kDa HSP) in myelin prepared from MS white matter and found that it stimulated a response in peripheral blood monocytes from MS patients that was not found in controls. Their results suggested that αB crystallin may be a prominent target of activated T cells in patients with MS.

The heat shock response is complex, and not necessarily pathogenic. Since HSP play an important role in protecting against environmental stress, their induction may provide a means through which healthy cells are shielded from damage near sites of inflammation or pathology. Also, HSP are important to the normal metabolism of the cell. HSC70 functions as a clathrin-uncoating ATPase, a microtubule-associated protein, and a molecular chaperone involved in the synthesis of a variety of proteins. We recently presented evidence consistent with a role for HSC70 as a chaperone for myelin

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basic protein (MBP) in oligodendrocytes (17). Most work on the small HSP, 27 kDa in humans (HSP27), has been performed in *Xenopus* and *Drosophila*, where it has been shown to play a role in regulating cell division and proliferation and is therefore important in embryogenesis (18, 19). We recently identified HSP27 in human fetal CNS tissue (20), where it may perform similar functions as well as play a role in depolymerizing actin microfilaments (21, 22).

It is unlikely that HSP are the primary or initiating antigens in MS, since in the majority of MS patients, prominent T- and B-cell responses are directed toward myelin antigens (23–25). However, an antibody response to HSP in MS lesions may contribute to the progression of disease. One can speculate that HSP involved in protective and/or regenerative mechanisms in nascent MS lesions may serve as antigens in the chronic plaque and eventually lead to tissue destruction (26). In Alzheimer disease and many other neurodegenerative disorders, elevated expression of HSP (particularly members of the 27-, 60- and 70-kDa families) has been reported; however, evidence of an immune response to HSP is not necessarily present in all cases (16, 27–30). This suggests that the expression and/or distribution of HSP in MS differs from that in other neurological diseases.

To gain additional insight into the role of HSP in MS, we have examined the distribution and level of expression of three HSP—HSC70, HSP70 and HSP27—in white matter and myelin isolated from MS lesions and compared them with normal CNS tissue.

## MATERIALS AND METHODS

### Tissue Acquisition

For immunocytochemistry, blocks of fresh-frozen CNS tissue obtained between 6 and 8 hours (h) postmortem were available from 4 cases of MS (one with acute/chronic active MS lesions, two with chronic active lesions, and one containing chronic silent lesions) and one normal subject. Tissue was embedded in optimum cooling temperature (OCT) medium, and frozen sections were cut at 8 to 10 μm. A total of 15 blocks were examined.

For quantitative analysis, blocks of fresh-frozen brain tissue from normal and MS cases were obtained from the National Neurological Research Specimen Bank (West Los Angeles VA Medical Center, Los Angeles, CA) (31). Each block represented a separate brain. Cryosections were prepared from these blocks, and each MS block containing white matter plaque material was classified according to immunological activity and degree of demyelination, as previously described (32). Four blocks of tissue were classified as containing "active" plaques, 4 blocks classified as containing "moderately active" plaques, and 8 blocks classified as containing "least active/inactive" plaques. Also, 4 blocks of tissue were obtained from histologically-determined "normal-appearing" white matter that was dissected from MS brains outside of lesion areas. As additional controls, 10 blocks were obtained from white matter regions of normal, non-MS brains. For comparison, 8 blocks of predominantly gray matter regions of normal, non-MS brains were obtained through the generosity of Dr Peter Davies (Department of Pathology, Albert Einstein College of Medicine). Postmortem times ranged from 6 to 23 h.

### Immunocytochemistry

Frozen sections of CNS tissue were air-dried, fixed in acetone for 10 minutes (min), washed, quenched with 0.03% $H_2O_2$, blocked with normal serum (dependent upon the species in which the secondary antibody was raised), incubated overnight at 4°C with monoclonal antibodies diluted in blocking solution, and stained using the avidin-biotin-peroxidase complex (ABC) technique (Vector Laboratories, Burlingame, CA) with 3,3'-diaminobenzidine (DAB) as chromogen. Two primary antibodies (Affinity BioReagents, Neshanic Station, NJ) were used; one reacts with both HSC70 and HSP70 (clone 3a3; 1:25), the other with HSP27 (clone G3.1; 1:100). These monoclonal antibodies worked optimally for immunostaining, and the ones listed below for immunoblotting. All are highly specific and well characterized (2, 3, 33, 34). For negative controls, primary antibodies were substituted by isotype-matched antibodies to irrelevant antigens.

### Quantitative Immunoblotting

Portions of frozen tissue from normal and immunocytochemically-classified MS white matter (0.2 to 0.6 g from each block) were homogenized in 8 M urea, and the content of HSC70 and HSP27 determined by quantitative immunoblotting (20, 35). Aliquots of each tissue homogenate were boiled in sample buffer containing 1% (wt/vol) SDS, resolved on SDS-PAGE alongside a set of purified standards at 4 to 5 different protein concentrations, and transferred to nitrocellulose membranes. Nitrocellulose membranes were blocked by incubation in 10% nonfat milk/0.1% Tween 20 in Tris-buffered saline (TBS), washed in TBS, and probed with monoclonal antibodies (StressGen, Victoria, BC) diluted in blocking solution. Three antibodies were used for immunoblotting analysis; one reacts with both HSC70 and HSP70 (clone N27F3-4; 1:3000), one with only HSP70 (clone C92F3A-5; 1:1000), and one with HSP27 (clone G3.1, as for immunocytochemistry; 1:1000). Nitrocellulose membranes were washed, incubated with the appropriate peroxidase-conjugated secondary antibody, washed again, and developed using an enhanced chemiluminescence (ECL) system (Amersham, Arlington Heights, IL). Immunoreactive bands were scanned by densitometry and a standard curve of purified HSC70 or HSP27 (StressGen) was constructed for each immunoblot. Sample volumes were adjusted so that the value for each tissue homogenate fell within the linear range of standard curves with correlation coefficients ≥ 0.98. The content of HSC70 and HSP27 was expressed as percent of total proteins in each tissue homogenate.

### Myelin Isolation, Protein Determination and Statistical Analysis

Myelin was isolated from blocks of white matter by the method of Norton and Poduslo (36), dissolved in 1% SDS, briefly sonicated, and analyzed by immunoblotting, as described above. Isolated myelin displayed a typical protein profile by

Coomassie blue staining of SDS gels, and its purity was confirmed by the lack (determined by immunoblotting) of several cytoplasmic proteins, including HSP27 and HSP70 in normal myelin, and GRP78 and GRP94 (stress proteins localized within the lumen of the endoplasmic reticulum) in normal and MS myelin. Antibodies to GRP78 (clone 10C3; 1:500) and GRP94 (clone 9G10; 1:1000) were obtained from StressGen. Protein content was determined by Modified Lowry Protein Assay (Pierce, Rockford, IL), using bovine serum albumin as the standard. Statistical significance was assessed by Student's t test, and p values less than 0.05 were deemed significant.

## RESULTS

### HSC70/HSP70 Immunoreactivity in MS Lesions

Frozen sections of CNS tissues from early autopsy MS cases were used to assess the expression of HSC70/HSP70 in MS lesions of different stages. In normal-appearing white matter, immunoreactivity for HSC70/HSP70 was prominent on nerve fibers, consistent with the high expression of HSC70/HSP70 detected in CNS tissue by quantitative immunoblotting (see below). In acute MS lesions, the lesion edge was clearly identifiable by a marked shift in the distribution of HSC70/HSP70 immunoreactivity from nerve fibers in the adjacent white matter to lipid-laden macrophages and scattered nerve fibers in the lesion center.

In chronic-active MS lesions, adjacent white matter showed intense staining of nerve fibers and myelin sheaths, whereas in the lesion center, immunoreactivity for HSC70/HSP70 on nerve fibers was markedly reduced (Fig. 1A). In addition, many axons within the lesion center showed dystrophic changes, characterized by focal dilatations that were strongly reactive for HSC70/HSP70 (Fig. 1B). Immunoreactivity was also noted on endothelial cells, perivascular macrophages, a few astrocytes, and numerous ramified microglial cells (Fig. 1B, C). In normal white matter, HSC70/HSP70 was widespread, with prominent expression on endothelial cells and astrocytes (Fig. 1D). In the center of chronic-silent lesions, immunoreactivity for HSC70/HSP70 was markedly diminished, except for a few scattered astrocytes that showed positive immunoreactivity (not shown).

### HSP27 Immunoreactivity in MS Lesions

Within the centers of chronic-active MS lesions, intense reactivity for HSP27 was noted on reactive astrocytes, some endothelial cells and a few round cells with the features of oligodendrocytes (Fig. 2A–E). At the lesion edge, immunoreactive cells of the latter type were detected in greater numbers, and were arranged in an interfascicular pattern, many of them displaying fine processes typical of oligodendrocytes (Fig. 2C, D). As seen for HSC70/HSP70, perivascular macrophages stained heavily for HSP27 (Fig. 2E). In white matter adjacent to the lesion, low-level reactivity was found on occasional endothelial cells and astrocytes (Fig. 2A), as in normal tissue (Fig. 2F). In chronic-silent lesions, the pattern of reactivity for HSP27 was more restricted to reactive fibrous astrocytes within the lesion center (not shown). This reactivity tapered off rapidly in adjacent normal-appearing white matter.

### Quantitative Immunoblotting for HSC70 and HSP27

Representative immunoblots of MS and normal white matter probed for HSC70, HSP70, and HSP27 are illustrated in Figure 3, and quantitative analysis of these data for HSC70 and HSP27 in Figure 4. Dramatically lower levels of HSC70 were present in plaque-containing material from MS white matter compared with either normal-appearing white matter or normal tissue (Fig. 3A). HSP70 was generally present in minor amounts relative to HSC70 (Fig. 3A), and no clear pattern of change and no statistically significant differences in HSP70 were detected between MS and normal tissue and among plaque types (Fig. 3B). For quantification, samples were analyzed on separate gels to augment resolution between HSC70 and HSP70. In white matter regions of normal human brain, HSC70 accounted for 1.64 ± 0.16% (mean ± SD) of the total protein content (Fig. 4A). In MS tissue, HSC70 represented 1.19 ± 0.10% of the total proteins in active plaques, 1.02 ± 0.21% in moderately active plaques, and 0.74 ± 0.13% in least active/inactive plaques (Fig. 4A). As lesions aged, less HSC70 was expressed. For example, the difference in HSC70 content between active and inactive plaques was found to be statistically significant by Student's t test, $p < 0.002$. Since HSC70 was present in myelin (see Figs. 1, 5), the loss of myelin probably accounted for the significant decreases in overall HSC70 content in MS white matter and the trend toward lower HSC70 content as lesions aged.

In contrast to HSC70, marked increases were noted in HSP27 expression (Fig. 3C). In the same samples examined for HSC70 content, HSP27 represented 0.024 ± 0.010% of the total protein content in white matter regions of normal tissue (Fig. 4B). In MS tissue containing active plaques, HSP27 represented 0.10 ± 0.03% of the total proteins, 0.060 ± 0.04% in moderately active plaques, and 0.074 ± 0.04% in least active/inactive plaques (Fig. 4B). Although HSP27 was elevated in all plaque types, no correlation could be made between plaque activity and HSP27 content.

Levels of HSC70, HSP70, and HSP27 in normal-appearing white matter from MS tissue were comparable to normal controls (Figs. 3, 4). However, normal white matter differed significantly from gray matter with respect to HSC70 and HSP27 content (Fig. 4). Predominantly gray matter regions of normal human brain contained more HSC70 (2.10 ± 0.54%) and less HSP27 (0.015 ± 0.007%) than normal white matter.

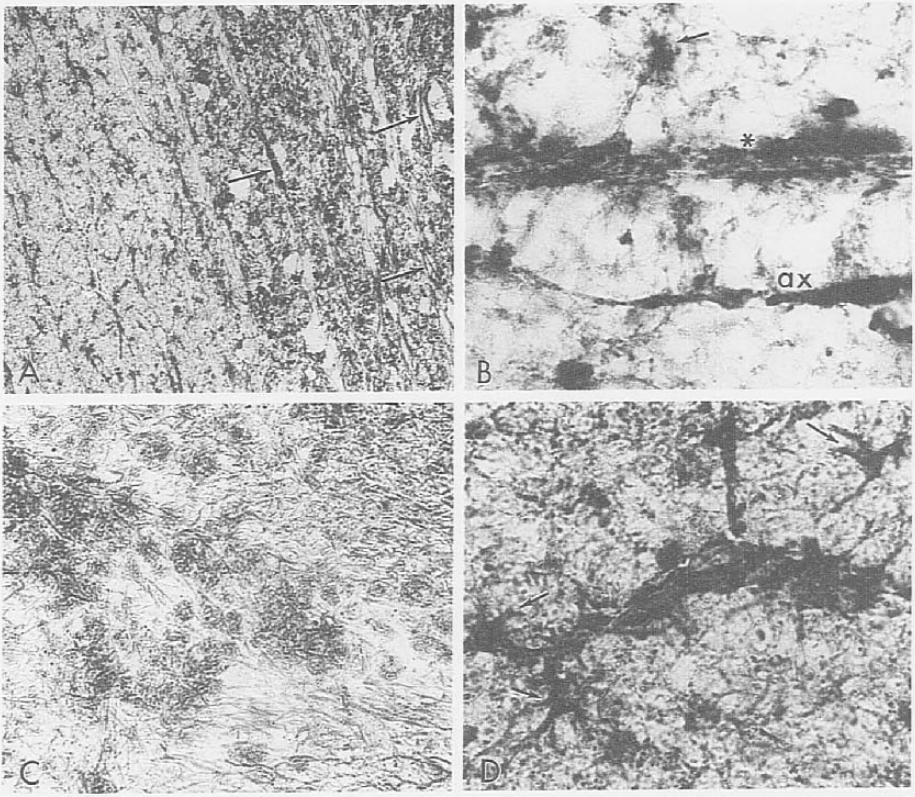


**Fig. 1.** HSC70 expression in MS and normal CNS tissue. (A) The edge of a chronic active MS lesion (lesion center toward left) shows positive reactivity on axons and blood vessels within the lesion and on myelin sheaths in the adjacent white matter (arrows). ×120. (B) Detail of the lesion area in (A) to show HSC70 immunoreactivity on astrocytes (arrow), endothelial cells of a small blood vessel (*), and a beaded, demyelinated axon (ax). ×750. (C) At the margin of a chronic active MS lesion, a perivascular cuff of HSC70⁺ macrophages is seen. Note also the twig-like background staining of fibrous astroglial cell processes, and in the adjacent white matter (upper right), some myelin staining. ×480. (D) HSC70 expression is shown in the white matter from a normal subject. Note the intense staining of the blood vessel (center) and many surrounding astrocytes (arrows). The background matrix shows a higher level of reactivity than in MS lesions (c.f. panel B). ×480.

### HSC70, HSP70 and HSP27 in Myelin

Myelin was isolated from 2 representative normal white matter samples (Fig. 5, lanes 1 and 2) and 4 active MS plaques (Fig. 5, lanes 3-6), and immunoblotted for HSC70/HSP70 (Fig. 5A) and HSP27 (Fig. 5B). Both normal myelin samples contained similar amounts of HSC70; however, no HSP70 or HSP27 was detected. HSC70 levels were increased approximately 4-fold in 3 out of 4 cases in myelin isolated from active MS lesions. Moreover, substantial amounts of HSP70 and HSP27 were found in MS myelin. HSC70 was consistently more abundant than HSP70 in MS myelin, and the variability in HSP levels among MS samples was possibly due to regional differences.

## DISCUSSION

In this study, we compared the distribution and expression of HSC70, HSP70 and HSP27 between normal and MS white matter by a combination of immunocytochemistry and quantitative immunoblotting. Striking

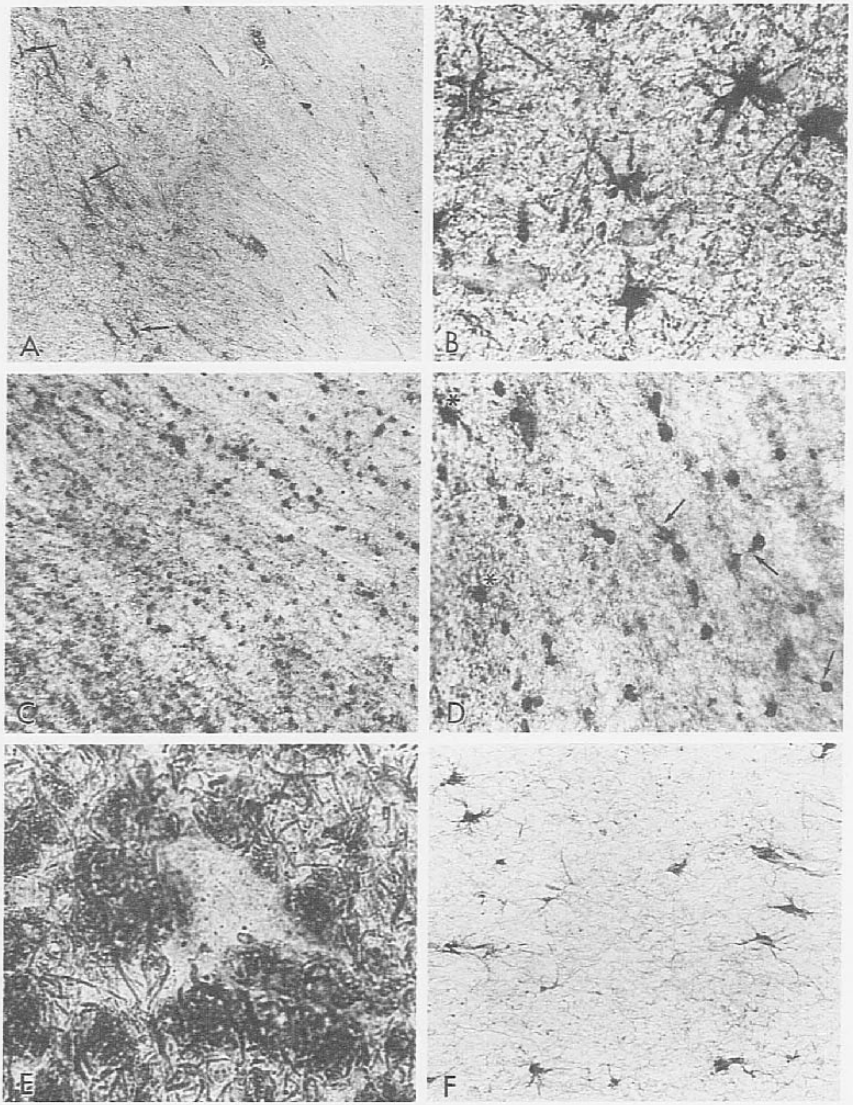


**Fig. 2.** HSP27 expression in MS and normal CNS tissue. (A) Fibrous astrocytes (arrows) within a chronic active MS lesion (left) display positivity for HSP27, while the adjacent myelinated white matter (right) shows little reactivity. ×75. (B) Within a chronic active MS lesion, numerous fibrous astrocytes display intense reactivity for HSP27. Note also the heavy staining of the background matrix material. ×480. (C) The myelinated edge of another chronic active MS lesion shows intense immunoreactivity for HSP27 on chains of interfascicular oligodendrocytes at numbers suggestive of hyperplasia. The lesion center is to the lower left. ×120. (D) Higher magnification of the same lesion in (C) to show the typical oligodendroglial morphology of the HSP27+

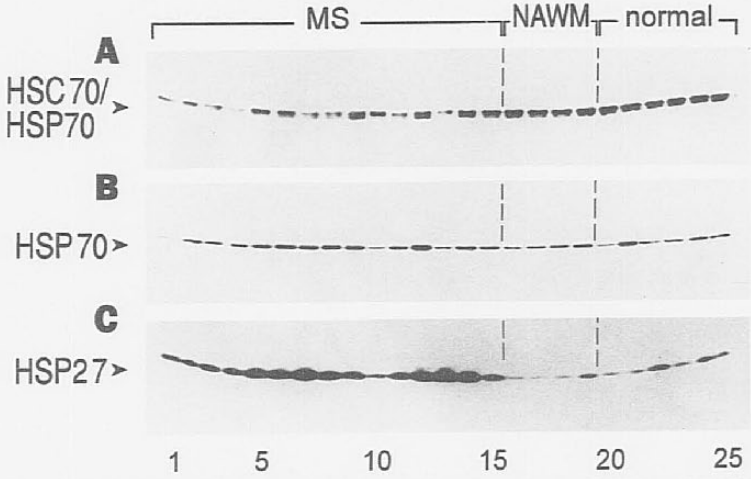


**Fig. 3.** Immunoblotting for HSC70, HSP70 and HSP27 in MS and normal white matter. To illustrate the differences in heat shock protein content in white matter from MS and normal cases, gels were loaded with equal amounts of total protein and immunoblots were probed with antibodies to (A) HSC70 (upper band)/HSP70 (lower band), (B) HSP70, and (C) HSP27. The following amounts of protein were loaded: (A) 5 μg; (B) 12 μg; and (C) 20 μg. Samples were loaded on each gel in the same order. Lanes 1 to 15 represent plaque-containing white matter from MS cases. Active plaques are illustrated in Lanes 3, 6 and 7; moderately active plaques in Lanes 1, 2, 5 and 14; and inactive plaques in Lanes 4, 8 to 13 and 15. Lanes 16 to 19 represent normal-appearing white matter (NAWM) from MS cases, and Lanes 20 to 25 represent white matter from normal subjects.

changes were noted in MS white matter, including: (a) significantly lower levels of HSC70 (30–50%); plaque regions were sharply defined by the loss of HSC70 relative to surrounding white matter; (b) significantly higher levels of HSP27 (2.5- to 4-fold); plaques that stained heavily for HSP27 and contrasted sharply with surrounding white matter; (c) increased expression of myelin-associated HSC70 (3- to 4-fold); and (d) prominent expression of myelin-associated HSP27 and HSP70, which were absent in normal myelin.

Immunocytochemical staining demonstrated that HSC70 was widely distributed in normal-appearing white matter (reflecting its constitutive expression in all cell types) and was present in myelin sheaths. However, HSC70 staining was dramatically reduced within plaques of all types, and decreasing levels of HSC70 as lesions aged were consistent with the loss of myelin. Within the active lesion, demyelinated axons were strongly positive for HSC70. In correlating the quantitative and immunocytochemical data, it appeared that even though a large proportion of HSC70 content was lost from lesioned white matter, levels of myelin-associated HSC70 were higher in the remaining myelin or in newly-synthesized myelin. Evidence of remyelination was present in these lesions, particularly at the edge of the lesion. Thus, it appeared that in MS white matter, although enhanced expression of HSC70 occurred in specific cellular components, overall losses were greater as the disease progressed. In contrast to HSC70, HSP27 content was increased in the same samples of MS white matter. Enhanced immunostaining for HSP27 was evident mostly in reactive astrocytes, myelin and hyperplastic interfascicular oligodendrocytes along the lesion edge. These $HSP27^+$ oligodendrocytes were arranged in linear arrays and clearly displayed the phenotype, i.e. round cell bodies and short, fine processes, distinctive of this cell type

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cells. Note the short, fine processes radiating from some of the cells (arrows). An occasional astrocyte (*) is also shown. Lesion center is to the left. ×300. (E) Perivascular macrophages in an acute MS lesion show heavy staining for HSP27, while the processes of fibrous astrocytes are somewhat less positive. ×480. (F) An area of white matter from a normal subject shows selective immunoreactivity for HSP27 on fibrous astrocytes and a very low background level of reactivity, in contrast to the situation in MS (c.f. panel B). ×300.

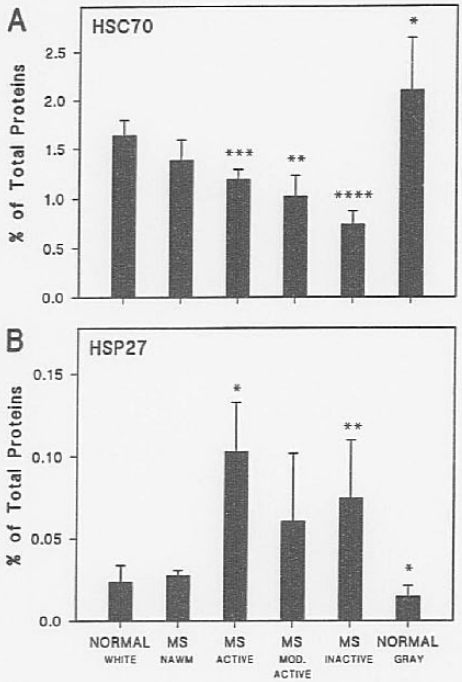


**Fig. 4.** Quantification of HSC70 and HSP27 in MS and normal white and gray matter. Levels of HSC70 (A) and HSP27 (B) in brain tissue were quantified by immunoblotting alongside a series of purified protein that was used to generate a standard curve, and expressed as percentage of total protein content in each sample. Data are reported as means + SD. Values that were found by Student's t test to be statistically different from normal white matter controls are noted: (A) * ($p < 0.05$), ** ($p < 0.005$), *** ($p < 0.002$), **** ($p < 0.0001$); (B) * ($p < 0.05$), ** ($p < 0.01$). NAWM: normal-appearing white matter from MS brains.

It is presently unclear why HSC70, HSP70 and HSP27 would be elevated in myelin isolated from active MS lesions; however, it is possible that these myelin-associated HSP may participate as part of a protective mechanism acting to prevent further destruction of the myelin sheath. In addition, we recently presented evidence consistent with a role of HSC70 as a chaperone for MBP (17). Additional work has shown that (a) MBP-HSC70 complexes exist in oligodendrocytes; and (b) denatured MBP associates into high molecular weight complexes with HSC70 during renaturation (Aquino et al, manuscript in preparation). Thus, increased levels of myelin-associated HSP may be necessary to aid in lesion repair by ensuring that extrinsic myelin proteins properly associate with the myelin membrane and by preventing proteolysis, aggregation and non-functional interactions during this process. On the other hand, this would suggest that decreased HSC70 content in MS white matter may contribute to demyelination or the lack of efficient and complete remyelination that is evident in MS, since under such circumstances myelin proteins may not be adequately chaperoned.

HSP27 is a chaperone as well (37, 38). It is interesting to note that HSP27 was present only in lesioned myelin and was prominent in oligodendrocytes at the lesion edge that are actively involved in the remyelination process. Unlike HSC70, which was present in normal myelin, HSP27 is probably not a primary chaperone of myelin proteins and is not likely involved in the maintenance of myelin, but may be recruited to the myelin sheath during times of stress or repair.

In this study, HSC70 and HSP27 levels were determined relative to the total protein content of homogenates prepared from normal and MS white matter containing plaques of different ages. By using purified HSC70 and HSP27 as standards, we were able to compare measurements from a large number of clinical samples since all were normalized against a common standard. Although in most cases statistical variation within each sample classification was minimal, that which was noted may be due to regional differences or varying amounts of normal-appearing tissue surrounding plaque areas that was taken during dissection. In previous studies using this same technique, we found levels of HSC70 and HSP27 in fetal human brain and adult rat spinal cord (3, 20) similar to those reported here for normal adult human brain. In the present study, we found that HSC70 content was greater in normal gray matter than in white matter, while the reverse was true for HSP27. In general this probably reflected a high expression of HSC70 among neuronal populations and a glial localization for HSP27, as has been reported (3, 20, 39, 40).

It is interesting to note that during the process of remyelination, additional (and possibly novel) immunogenic epitopes may be expressed in myelin. This has been demonstrated in the detection of an elevated T cell response to exon 2 of MBP in patients with MS (41). Exon 2-containing MBP isoforms are relatively minor in the adult, yet their expression is high during developmental myelin formation (42) and during remyelination in MS (43). As in myelinogenesis, chaperones should be similarly required for remyelination during the process of lesion repair in MS. However, enhanced membrane expression of these HSP positions them as potential targets of the immune response and consequently may contribute to the progression of chronic MS. Such has been proposed for αB-crystallin, a small HSP, which is not present in normal myelin but is present in myelin isolated from

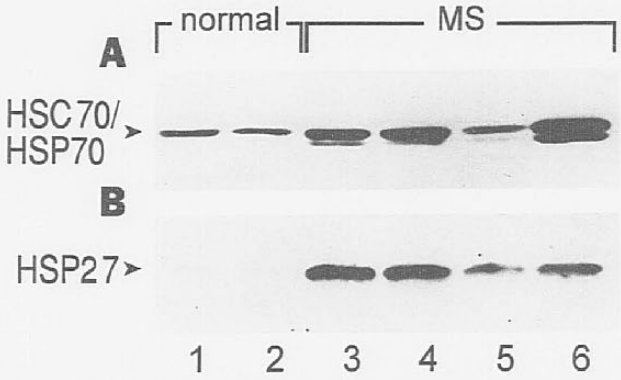


**Fig. 5.** HSC70, HSP70 and HSP27 in myelin. Myelin was purified from 2 separate samples of human white matter from normal subjects (Lanes 1 and 2) and 4 separate samples of MS white matter containing active plaques (Lanes 3 to 6). Myelin proteins were resolved by SDS-PAGE and immunoblotted for (A) HSC70 (upper band)/HSP70 (lower band) and (B) HSP27. Equal amounts of myelin proteins were loaded per lane in each panel, 40 μg for HSC70/HSP70 and 90 μg for HSP27.

MS tissue (16). Although HSP alone may serve as the immune targets, it is also possible that novel antigens may result from the association of a chaperone and its substrate (e.g. HSC70 and MBP, respectively). Both HSC70 and MBP are highly immunogenic (15).

In conclusion, we found that white matter undergoing immune-mediated destruction was associated with an altered distribution and expression of HSC70 and HSP27. The factors responsible for these changes are unknown. However, cytokines are likely candidates since both IL-1 and TNFα are found at high levels in MS lesions (44, 45) and have been shown to induce synthesis of HSP in cells of the CNS (15, 46). Furthermore, our findings that HSC70, HSP70 and HSP27 were present in myelin isolated from lesioned white matter in MS are novel and contribute to the growing interest in the role of HSP in the pathogenesis of this disease. It is intriguing to speculate that these HSP, which initially may function to protect myelin from damage and aid in its regeneration, eventually may serve as additional targets of the immune response and contribute to the progression of chronic MS.

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