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Association of MBP peptides with Hsp70 in normal appearing human white matter

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Abstract

Multiple Sclerosis is an autoimmune disease directed against myelin proteins. The etiology of MS is poorly defined though, with no definitive causative agent yet identified. It has been hypothesized that MS may be a multifactorial disease resulting in the same end product: the destruction of myelin by the immune system. In this report we describe a potential role for heat shock proteins in the pathogenesis of MS. We isolated Hsp70 from the normal appearing white matter of both MS and normal human brain and found this was actively associated with, among other things, immunodominant MBP peptides. Hsp70–MBP peptide complexes prepared in vitro were shown to be highly immunogenic, with adjuvant-like effects stimulating MBP peptide-specific T cell lines to respond to normally sub-optimal concentrations of peptide. This demonstration of a specific interaction between Hsp70 and different MBP peptides, coupled with the adjuvanticity of this association is suggestive of a possible role for Hsp70 in the immunopathology associated with MS.

Keywords: Multiple Sclerosis; Hsp70; MBP; Immune response; Adjuvant; Autoimmune

1. Introduction

MS is a degenerative demyelinating disease of the central nervous system, the pathology of which demonstrates a predilection for certain regions such as optic nerve, periventricular regions, brainstem, and spinal cord, but which has become increasingly recognized as a global disease of the neuraxis [1–4]. The etiology of MS is still not defined, though the strongest evidence suggests that MS, at least when it presents clinically, is an autoimmune disease which results from the cumulative effects of multiple factors including molecular mimics, genetic determinants and multiple environmental influences [2,3,5–8]. Thus in MS, myelin antigens such as MBP, PLP, MOG and myelin-associated glycoprotein, and non-myelin antigens such as αβ-crystallin, transaldolase and CNPase, are believed to become the targets of pro-inflammatory, pathogenic T cells [4,9–16]. How these protein antigens are first presented to the immune system and how this leads to the pathogenesis of MS is however not known.

Heat shock proteins (HSP) are the most abundant of all soluble intracellular proteins and play an important role in cell and organism survival through their ability to interact with a wide range of proteins and peptides [17–19]. There are over ten different families of human HSP with most active research focused on the families of Hsp90, Hsp70, Hsp60 and small heat shock proteins [20–23]. Under normal

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conditions all HSP participate in intracellular protein folding and chaperoning [18,24]. However, following an insult such as heat shock, viral infection or trauma, most HSP protein synthesis is upregulated and these proteins are variably translocated throughout the cell and secreted into the exterior milieu [18,24]. At this stage the role of HSP becomes one of the protein protection and stabilizing, thus enabling proteins to retain function and facilitate the repair of the cell [18,24].

In the 1980s HSP were identified as tumor rejection antigens, the antigenic moiety of HSP being the associated tumor antigens and not the HSP itself [25–28]. In vitro reconstituted HSP–tumor peptide complexes were shown to stimulate tumor-specific immune responses, and it is now well established that HSP–peptide complexes are released from stressed cells [29–32]. These HSP–peptide complexes are capable of stimulating the maturation of professional antigen presenting cells (APC) [33,34] most likely through their interaction with candidate receptors such as CD91, toll-like receptor-2 (TLR2), TLR4 or CD40 [35–37]. Following internalization, the HSP-associated peptides are processed by the APC and presented to the immune system via MHC class I or class II molecules [38,39]. HSP–peptide complexes can be reconstituted in vitro [29,40,41] and Hsp70–peptide complexes were shown to stimulate peptide-specific T cells responses that were significantly greater than the response to peptide or protein alone [39,42]. This immunostimulatory and adjuvant nature of HSP association with peptides or proteins has been successfully exploited in the treatment of experimental tumor models [30,43] and in clinical trials as a cancer therapy [44,45].

In this study we have focused on the role Hsp70, one of the most widely upregulated HSP in response to stress [21]. Hsp70 has a two-domain structure which is critical for its known functions, a ~44 kDa N-terminal nucleotide binding domain that binds and catalyzes the hydrolysis of ATP and a ~27 kDa C-terminal substrate binding domain that interacts with polypeptide targets [46,47]. The activities of these two domains are coupled with a linker that alters the substrate domains affinity for peptide when ATP binds [48]. The affinity of Hsp70 for peptide targets is increased upon hydrolysis of ATP to ADP, and substrate binding can further stimulate ATP hydrolysis [21,49].

Many different HSP have been implicated in the pathogenesis of MS from their observed expression in and around MS lesions [16,50,51] to the detection of increased immune responses against HSP [52,53]. Indeed, glial cells, astrocytes, neurons and oligodendrocytes all possess the ability to express HSP following stress [54–56]. Although research has suggested the importance of HSP in the expression of myelin proteins [57], little importance has been given to any protein or peptide moieties that may be associated with these upregulated HSP following the cellular stresses associated with MS. It is only recently that researchers have suggested a potential role for the association of HSP with whole myelin proteins in the modulation of the immune response to myelin [39,58].

We have proposed that the 'adjuvanticity' of HSP association with peptide occurs in the "stressed" brain and that when this occurs in individuals with a susceptible genetic background this could lead to the initiation of myelin-specific immune responses in MS. In this study we show that peptides derived from MBP are the major detectable moieties found associated with Hsp70 in post-mortem, normal appearing white matter both from MS brain and normal brain. We further show that this association of MBP peptides with human Hsp70 causes an adjuvant-like effect resulting in significantly enhanced responses by peptide-specific T cells to otherwise non-stimulatory concentrations of MBP peptides.

2. Materials and methods

2.1. Human samples

The protocol for this study was approved by the USC Institutional Review Board. All human brain specimens were obtained from the Human Brain and Spinal Fluid Resource Center, VAMC, Los Angeles, California 90073, which is sponsored by NINDS/NIMH, National Multiple Sclerosis Society, VA Greater Los Angeles Healthcare System and Veterans Health Services and Research Administration, Department of Veteran Affairs. Snap frozen slices of normal appearing white matter were obtained from regions directly adjacent to demyelinated plaques in deceased subjects who had been definitively diagnosed with Multiple Sclerosis prior to their death. As a control, we also obtained normal appearing white matter from deceased subjects who had no known neurological or autoimmune diseases at the time of their death. All materials were de-identified with autolysis time varying from 7 to 15 h.

To generate antigen-specific T cell lines, blood was collected from eight [8] healthy volunteers, all of whom gave their informed consent to participate in this study. Following collection of the whole blood, PBMC were isolated by density centrifugation over Ficol-Paque (Pharmacia, Piscataway, NJ) and used as described in T cell line preparation.

2.2. Purification of Hsp70 and elution of associated moieties

Hsp70 was immunoprecipitated from homogenates of human white matter according to commonly used procedures, and the associated peptides and proteins eluted according to the published method of Ishii et al., with minor modifications [28]. Unless otherwise stated, all chemicals were obtained from VWR International, PA. Frozen white matter (5–10 g) was homogenized in 4 vol. of ice cold hypotonic buffer (10 mM $NaHCO_3$, 0.5 mM PMSF pH 7.1 (Sigma-Aldrich, MO)), centrifuged at 100,000 g for 3 h at 4 °C, and the clarified supernatant collected. The white matter supernatant was then incubated overnight at 4 °C with a monoclonal antibody specific for Hsp70 coupled to