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Response to Comment on "Chaperone Activity of α B-Crystallin Is Responsible for Its Incorrect Assignment as an Autoantigen in Multiple Sclerosis"

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Letters to the Editor

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Comment on "Chaperone Activity of α B-Crystallin Is Responsible for Its Incorrect Assignment as an Autoantigen in Multiple Sclerosis"

We have read with interest the recent paper by Rothbard et al. (1), who suggest that small heat shock proteins (HSPs), including α B-crystallin, bind to human Abs as molecular chaperones rather than being recognized as an Ag. The authors state that this has led to the incorrect assignment of α B-crystallin as an autoantigen in multiple sclerosis (MS). However, the original assignment (2) was based on the finding that α B-crystallin is the dominant myelin-associated activator of human T cells, when it accumulates in the oligodendrocyte/myelin unit to levels seen in MS patients. This accumulation, and its relevance for activation of T cells at very early stages of MS lesions, was confirmed by several follow-up studies (3–7). Thus, the ability of α B-crystallin to activate T cells has always been the basis for its assignment as an autoantigen in MS, not because it would be a target for Abs.

Still, human serum Abs do bind to α B-crystallin and, importantly, do so in ways that contradict the claim by Rothbard and coworkers (1). The chaperone activity of α B-crystallin and other small HSPs is critically dependent on the formation of oligomers, dimers at least (8–10). Peptide fragments or monomers of α B-crystallin are therefore unable to act as chaperones. Yet, human Abs recognize different 20-mer peptide fragments of α B-crystallin on microchips (11) and monomeric human α B-crystallin on Western blots (7, 12, 13). These findings should have prevented the authors from dismissing the protein as a bona fide Ag for human Abs, let alone as the main myelin-associated activator of T cells.

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The comments of Dr. van Noort and colleagues in regard to our manuscript are appreciated because they allow us to discuss an important issue that was not included in the published article.

The goal of the research was not to question whether crystallin was an autoantigen, but rather to address the perceived problem that preexisting Abs present to a potential therapeutic protein. However, when Igs from the sera of 27 multiple sclerosis patients were shown to bind each member of a set of eight human small heat shock proteins (sHsps), we questioned whether such cross-reactive Abs existed. When an identical pattern of binding was observed using sera of 13 normal individuals, we realized that sHsps binding the Abs in serum could explain the phenomenon. Further experiments

described in the article demonstrate that all members of the human sHsp family have the capacity to bind Abs, and consequently positive results in simple ELISA assays could arise either from the Ab binding the Ag, or the reverse, making interpretation difficult.

Dr. van Noort makes an important point by stating that Abs to crystallin must exist based on interactions observed using Western blots and peptide arrays. We were conscious of these data, having published some of the results ourselves, and have investigated this question. Several groups have established that crystallin peptides have the capacity to bind a variety of proteins and exhibit chaperone activity. In a series of papers, Ghosh and Clark (1–3) have defined regions with HspB5 that bind a variety of intracellular proteins and cytoskeletal elements, whereas Sharma and colleagues (4, 5) have demonstrated that residues 72–93 of both HspB4 and B5 exhibit chaperone activity equivalent to that of the parent protein. We have confirmed the chaperone activity of the 72–93 residues and extended it to include HspB1. The ability of the peptides to act as chaperones indicates that partially unfolded forms of the protein, which would be expected to be present in Western blots, are also capable of binding Abs. Consequently, binding in these assays also is inherently ambiguous and cannot be used as support for the presence of anti-sHsp Abs. The regions binding Abs in the arrays included are, as shown by Ghosh and Clark (1–3), capable of binding proteins as well. We are currently exploring the mode of action of the different peptides and examining whether they could be therapeutic in animal models of inflammation.

Our studies have not focused on T cell responses, but as stated in the manuscript, two separate groups have established that α B-crystallin–specific T cells are not pathogenic and do not induce EAE. Rather than argue about the semantics of the definition of an autoantigen, we believe our results should motivate the reinvestigation of the earlier studies. If, as we have established, α B-crystallin binds Abs in solution, they also have the potential to crosslink Abs, or other Ab family members, on the surface of cells and nonspecifically induce lymphocyte proliferation. Such a result could explain the apparent discrepancies in the literature that van Noort and colleagues have emphasized.

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