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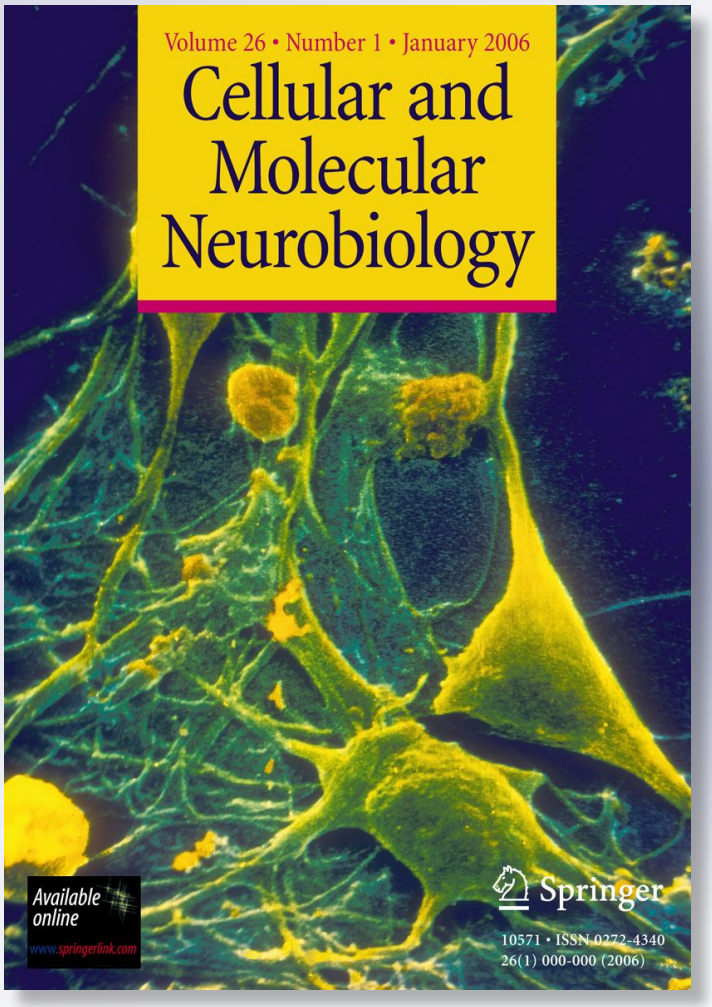

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SHORT COMMUNICATION

The Molecular Anatomy of Human Hsp60 and its Similarity with that of Bacterial Orthologs and Acetylcholine Receptor Reveal a Potential Pathogenetic Role of Anti-Chaperonin Immunity in Myasthenia Gravis

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Abstract Heat-shock protein 60 (Hsp60) is ubiquitous and highly conserved being present in eukaryotes and prokaryotes, including pathogens. This chaperonin, although typically a mitochondrial protein, can also be found in other intracellular sites, extracellularly, and in circulation. Thus, it can signal the immune system and participate in the development of inflammation and immune reactions. Both phenomena can be elicited by human and foreign Hsp60 (e.g., bacterial GroEL), when released into the blood by infectious agents. Consequently, all these Hsp60 proteins become part of a complex autoimmune response characterized by multiple cross reactions because of their structural similarities. In this study, we demonstrate that Hsp60 proteins from humans and two common pathogens, *Chlamydia trachomatis* and *Chlamydia pneumoniae*, share various sequence segments of potentially highly immunogenic epitopes with acetylcholine receptor α1 subunit (AChRα1). The structural data indicate that AChRα1 antibodies, implicated in the pathogenesis of myasthenia gravis, could very well be elicited and/or maintained by self- and/or bacterial Hsp60.

Keywords Shared epitopes · Molecular mimicry · Autoimmunity · Neuromuscular disorder

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Introduction

Unfolded or misfolded proteins may interfere with the machinery of the cell. For this reason, prokaryotic and eukaryotic cells have evolved special macromolecular "chaperone" complexes that capture and refold partially folded proteins (Ellis 2007). First observed in cells exposed to high temperatures, heat shock proteins (Hsps) have been identified as chaperones and found to increase in response to a number of cell stress stimuli (Haak and Kregel 2008). Chaperonins, a subset of chaperones, are classified in two groups: those belonging to Group I are found in bacteria (GroEL) and eukaryotic organelles (mitochondrial Hsp60, often called also Cpn60), while Group II chaperonins are present in archaea (thermosome subunits) and eukaryotic cytoplasm (CCT) (Macario et al. 1999).

The mitochondrial Hsp60 (hereinafter "Hsp60") is one of the most conserved human molecular chaperones and is also present in several kinds of pathogens (Sakamoto and Ohkuma 2010). In eukaryotic cells, this protein is classically considered an intracellular chaperone, working together with its co-chaperonin Hsp10 to assist in the folding of other mitochondrial proteins (Horwich et al. 2007; Lund 2009). Hsp60 has also been found in extra-mitochondrial

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sites, as well as in the extracellular environment and bloodstream, and thus can stimulate the innate and the adaptive immune systems (Habich and Burkart 2007; Henderson 2010; Quintana and Cohen 2011). Hsp60 activates macrophages and dendritic cells, stimulates production of pro-inflammatory molecules, and leads to activation of Hsp60-specific T cell responses. Moreover, both microbial and human Hsp60 are strongly antigenic and can trigger the production of relatively large amounts of antibodies. Not surprisingly, autoantibodies to self-Hsp60 are detected in several autoimmune and inflammatory diseases (Quintana and Cohen 2011).

Myasthenia gravis (MG) is a neuromuscular disorder caused by a T-cell-dependent and B-cell-mediated autoimmune response to the muscle-type nicotinic acetylcholine receptor (AChR), which compromises neuromuscular transmission (Moiola et al. 1998; Hohlfeld and Wekerle 2008; Le Panse et al. 2008; Helgeland et al. 2011). The crystal structure of AChR reveals an allosteric membrane glycoprotein constituted of five subunits (two α1 subunits, one β, one δ, one γ for embryonic stage, and one ε subunit for adult stage). For each subunit, extracellular, transmembrane, and cytoplasmic domains can be distinguished. The extracellular α1 subunits' domain contains, at the apex, the main immunogenic region (MIR), comprising residues 67–76 called "the MIR loop" and, half way up at the $\alpha\delta$ subunit interface, the high-affinity acetylcholine (ACh)-binding site called loop C, shaped by residues Y^{190}, C^{192}, C^{193}, and Y^{198} (Arias 2000). The MIR is a conformation-dependent region with immunogenic relevance for its prominent location, acting as a antigenic epitope in MG (Brejc et al. 2001). The presence of this region might allow native AChRs to be effective in binding to receptor immunoglobulins on the surface of pre-B lymphocytes (Lindstrom et al. 2008). Several experiments, carried out by creating chimeras of human α1 subunits, have demonstrated that half or more of the autoantibodies to AChR in MG are directed against MIR, thus confirming its prominent pathogenetic role (Lindstrom et al. 2008; Luo et al. 2009). Moreover, the presence of the α1 MIR sequence in chimeric AChR greatly influences its sensitivity to activation by Ach (Luo et al. 2009). In α1 subunits, conformation maturation of the MIR and the ACh-binding site occurs simultaneously before assembly, revealing the structural and functional significance of the former (Brejc et al. 2001; Luo et al. 2009).

The question is: How do anti-AChR antibodies arise? We previously reported several highly homologous amino-acid sequences when comparing human and *Chlamydial* Hsp60 with various subunits of human AChRs (Cappello et al. 2010). In the present study, we extended our search to all the homologous epitopes that result from the comparison between the amino-acid sequences of AChRα1 and *Homo sapiens* Hsp60 (Hs-Hsp60), or *Chlamydia trachomatis* GroEL (Ct-Hsp60), or *Chlamydia pneumoniae* GroEL (Cp-Hsp60). We also mapped these epitopes within the three-dimensional structure of the AChRα1 molecule with particular attention to the MIR and the ACh-binding site. Our results support the hypothesis of cross-reactivity between human and *Chlamydial* Hsp60s with AChR, which would lead to a humoral anti-Hsp60 immune response that would also react with AChR and, thus, contribute to the pathogenesis of MG.

Methods

The amino-acid sequences of AChRα1, Hs-Hsp60, Ct-Hsp60, and Cp-Hsp60 were obtained from the PubMed website (http://www.ncbi.nlm.nih.gov/genbank/) using the access numbers Y00762, NM002156, AJ783840, and AAP98858, respectively. Sequence comparisons were performed using the SIM software (http://expasy.org/tools/sim-prot.html), and a three-dimensional model of AChRα1 monomer was obtained using the 3D-JIGSAW software

Table 1 Sequence segments of high similarity in human and chlamydial Hsp60 proteins shared with AChRα1

Hsp60 amino-acid sequences compared to AChRα1 from	Amino acid position	Percent similarity	Extracellular AChRα1 epitope
Homo sapiens			
LIVEKIMQSSSEV	489–501	53.8	No
FEKISKGANPVEIRRG	128–143	38.9	Yes
VIAELKKQSKPVTTPEE	155–171	35.3	Yes
AIATGGAGEE	317–326	33.3	Yes
Chlamydia trachomatis			
AGLLLTTE	512–519	62.5	Yes
GGVAVIRV	373–380	50.0	Yes
NIKYNEEARKKIQK	5–18	28.6	Yes
IKYNEEARKKIQKGVKTL	6–23	27.6	Yes
Chlamydia pneumoniae			
SEQEKLSNY	2–10	55.6	Yes
SEQNQHLIIFCEDID	235–249	53.3	No
DGDAVIAKLSSL	448–459	41.7	Yes
IVKGSYGPKQSLS	26–38	38.5	Yes
YNADKKLFS GIDKLFQIVK	10–28	36.8	Yes
LGVDFAKAMVNK IHKEHS	63–80	33.3	Yes
YENLGVDFAKAMV NKIHKEHSDGAT	60–84	28.0	Yes
PKQSLSPTSFFKE RGFYAISQT	33–54	27.3	Yes
LQEALQQQSWPIKDA	121–135	26.7	Yes
LHAILQESYAALEKGIS THKLIASLKLQGEKLQ	90–122	18.2	Yes

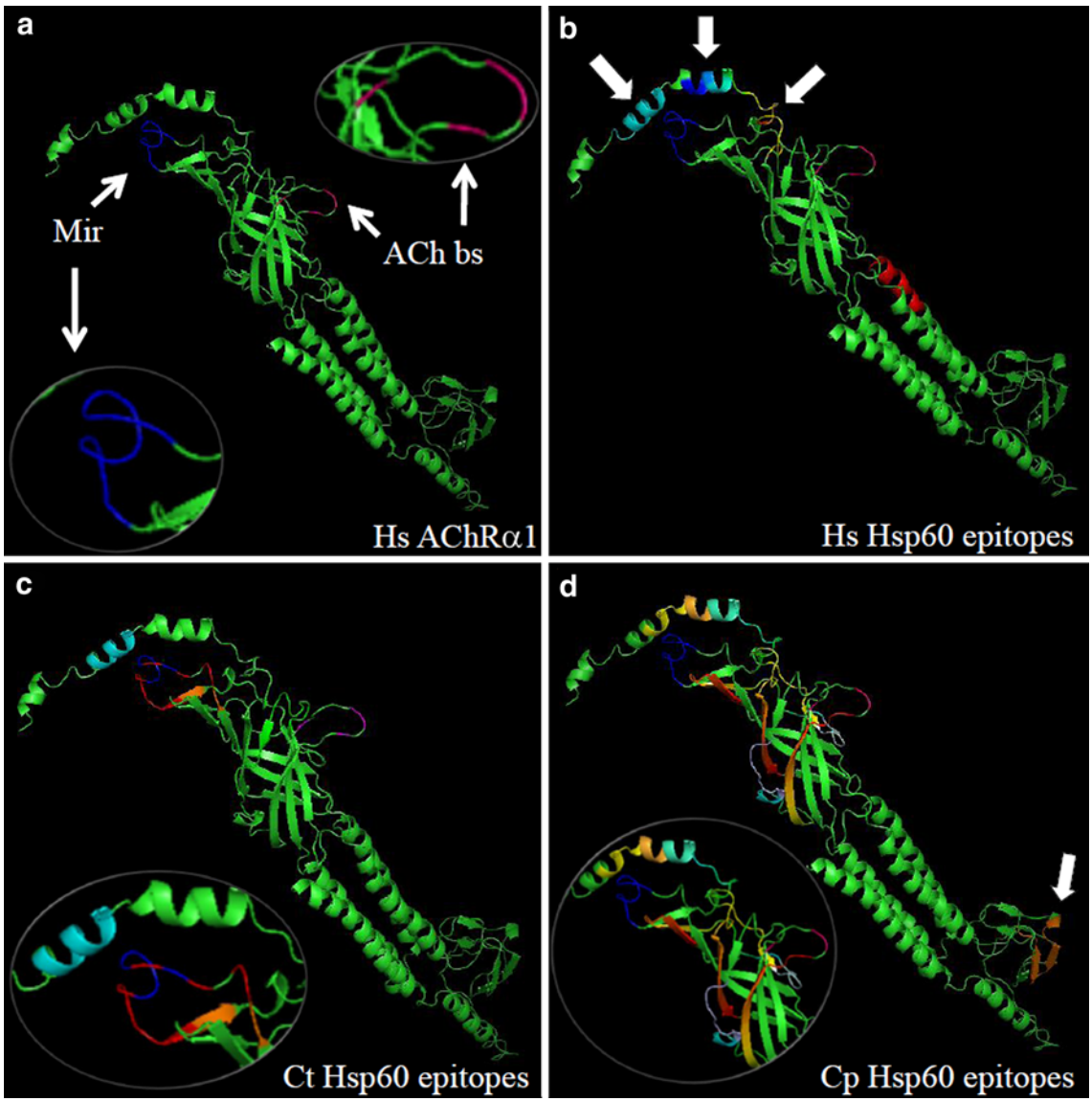

Fig. 1 Three-dimensional model of AChRα1 monomer showing shared amino-acid sequences (potential immunogenic-antigenic epitopes) with the Hs-Hsp60 and its chlamydial counterparts. **a** Shown are the Mir (*blue loop*, *bottom left corner insert*) and the ACh-binding site (ACh-bs; *hot pink discontinuous loop*, *top right corner insert*). **b** Homologous epitopes revealed by the alignment of AChRα1 and Hs-Hsp60 sequences. One epitope maps to the transmembrane region of AChRα1 (*red helix*, aa 489-501), while another three epitopes (shown by *white thick arrows*) map to around the Mir. **c** Homologous epitopes revealed by the alignment of AChRα1 and Ct-Hsp60 sequences. Four epitopes (*cyan helix* and *red loops*—see *bottom left corner insert*) map around and on to the Mir. **d** Homologous epitopes revealed by the alignment of AChRα1 and Cp-Hsp60 sequences. Of the 10 shared sequences, only one (shown by a *white arrow*, aa 235–249) maps to the intracellular region of AChRα1, whereas all the rest (various colors; see insert down to the left) map around and on the ACh-binding site (color figure online)

(http://bmm.cancerresearchuk.org/~3djigsaw/) and visualized by PyMol (http://www.pymol.org/). All software used are open access.

Results and Discussion

The alignment of the amino-acid sequences of Hs-Hsp60 and AChRα1 revealed four epitopes with a percentage of similarity (identities plus conservative substitutions) comprised between 33.3 and 53.8 (Table 1). Likewise, the alignment of the amino-acid sequences of Ct-Hsp60 and AChRα1 showed four homologous epitopes with a similarity percentage comprised between 27.6 and 62.5. The comparison of the amino-acid sequences of Cp-Hsp60 and AChRα1 revealed 10 homologous epitopes with a percentage of similarity comprised between 18.2 and 55.6. Interestingly, these epitopes were mostly localized around

or inside either the MIR or the ACh-binding site of AChRα1 (Fig. 1).

Hsp60 of pathogenic eukaryotes and prokaryotes share numerous highly homologous (if not identical) amino-acid sequences distributed along the entire Hsp60 sequence with the potential of eliciting, by molecular mimicry, the production of cross-reactive antibodies after infection with these pathogens (Cappello et al. 2009). For example, GroEL of *Chlamydiaceae* share several epitopes with Hs-Hsp60 (Campanella et al. 2009). Thus, chlamydial Hsp60 represents a common dominant bacterial antigen, and immune responses to this pathogen are characterized by the production of antibodies to it (Cappello et al. 2009). As a consequence, the potential cross-reactivity between the microbial chaperonin and its human counterpart may predispose to autoimmunity. Recurrent infections and the ensuing prolonged antibody response may cause the exacerbation of an underlying autoimmune disease. In addition, enhanced expression of Hs-Hsp60 under stress conditions can also unveil previously silent antigenic determinants that can initiate and perpetuate autoimmune reactivity (Quintana and Cohen 2011).

There is extensive information about the association between autoantibodies to Hs-Hsp60 and the development of various autoimmune and inflammatory diseases, including type 1 diabetes, rheumatoid arthritis, multiple sclerosis, lupus, atherosclerosis, Behçet's disease, and inflammatory bowel disease (Quintana and Cohen 2011). However, very little is known about the humoral immune response to Hsp60 in MG, while the clinical association between chlamydial infection and MG has not been examined before. By contrast, the involvement of molecular mimicry for antigens from other bacterial infections (like *M. pnuemoniae* and *S. thyphimurium*) has been already postulated (Deitiker et al. 2000).

In this study, we found several potentially immunogenic epitopes that are located in the extracellular region of AChRα1, around and inside the MIR or the ACh-binding site, that are also present in Hsp60. These results strongly support the notion that human and bacterial Hsp60 might play a key role in the development of an autoimmune response against AChRα1. Thus, AChRα1 would become target for antibodies elicited by self- or foreign Hsp60, for example, during a chlamydial infection. If so, myasthenia gravis should also be considered a chaperonopathy (Macario et al. 2010), in which normal chaperones play a role in pathogenesis rather than the reverse, and therapeutic efforts should include blocking the chaperonins or their antibodies, or both.

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