

## Serum levels of antibodies to *Chlamydia pneumoniae* and human HSP60 in giant cell arteritis patients

M. López-Hoyos<sup>1</sup>, L. Álvarez<sup>2</sup>,  
M. Ruiz Soto<sup>2</sup>, R. Blanco<sup>2</sup>,  
M. José Bartolomé<sup>1</sup>,  
V.M. Martínez-Taboada<sup>2</sup>

Divisions of <sup>1</sup>Immunology and  
<sup>2</sup>Rheumatology, Hospital Universitario  
Marqués de Valdecilla; Facultad de  
Medicina-Universidad de Cantabria;  
Fundación Marqués de Valdecilla-IFIMAV,  
Santander, Spain.

Marcos López-Hoyos, MD  
Lorena Álvarez, pre-doctoral student  
María Ruiz Soto, pre-doctoral student  
Ricardo Blanco, MD  
María José Bartolomé, MD  
Victor M. Martínez-Taboada, MD

This work was partially supported by  
grants from the Fundación Marqués de  
Valdecilla-IFIMAV to Dr. M. Ruiz Soto  
and Fundación Mutua Madrileña.  
Dr. L. Álvarez was supported by a grant  
for research aid from Wyeth Pharma,  
Spain.

Please address correspondence and  
reprint requests to:  
Marcos López-Hoyos,  
Division of Immunology,  
Hospital Universitario Marqués de Valdecilla,  
39008 Santander, Spain.  
E-mail: inmlhm@humv.es

Received on January 2, 2007; accepted in  
revised form on June 13, 2008.

© Copyright CLINICAL AND  
EXPERIMENTAL RHEUMATOLOGY 2008.

**Key words:** *Chlamydia pneumoniae*,  
HSP60, giant cell arteritis,  
polymyalgia rheumatica, antibodies.

## SUMMARY

**Objective.** To measure the serum levels of IgG anti-*Chlamydia pneumoniae* (*C. pneumoniae*) and human heat shock protein (hHSP) 60 antibodies in patients with active giant cell arteritis (GCA) and to determine whether such levels decrease with corticosteroid therapy and remission of symptoms.

**Methods.** IgG anti-*C. pneumoniae* and anti-hHSP60 antibodies were quantified by commercial and in-house ELISA tests, respectively, in serum samples from 17 GCA patients, 39 polymyalgia rheumatica (PMR) patients and 23 age-matched healthy subjects.

**Results.** Serum IgG anti-hHSP60, but not anti-*C. pneumoniae*, antibodies were significantly increased in GCA patients in comparison with PMR patients or healthy controls. After steroid therapy, both anti-hHSP60 and -*C. pneumoniae* antibodies decreased significantly in both groups of patients.

**Conclusion.** Although some infectious agents have been suggested to participate in GCA pathogenesis, our data do not suggest that *C. pneumoniae* might be one of them. The production of anti-hHSP60 antibodies is shared in GCA with other systemic diseases and may be triggered by unknown infectious agents and/or other inflammatory factors.

## Introduction

Giant cell arteritis (GCA) is a granulomatous vasculitis affecting medium and large-size arteries in elderly people (1-3). The aetiopathogenesis of this disorder remains unknown although genetic, autoimmune and environmental factors have been implicated (4). GCA involves an inflammatory process in which infections are considered as possible pathogenic factors. Among the number of infectious agents implicated in GCA, *Chlamydia pneumoniae* (*C. pneumoniae*) is the one that has shown a closest association. In this regard, several reports including small numbers of patients identified DNA encoding *C. pneumoniae* (5, 6). Nonetheless, two well-designed studies in different cohorts of GCA patients have been published (7, 8), where they conclude that *C. pneumoniae* does not play an important role in the etiology of GCA.

*C. pneumoniae* is a gram-negative bacterium that replicates intracellularly and expresses several proteins, among which major outer membrane protein (MOMP) and chlamydial heat shock protein (cHSP) 60 are better known. Regan *et al.* (7) found only one GCA patient positive for the MOMP gene from a large sample consisting of 90 GCA patients. The same proportion was found for healthy controls. The Authors addressed such a study because of the similarities between GCA and atherosclerosis, where the pathogenic role of *C. pneumoniae* seems to be better established (9). In addition, Helweg-Larsen *et al.* (8), undertook a similar study on temporal artery biopsies from 15 GCA and 10 polymyalgia rheumatica (PMR) patients and also found no evidence of DNA from *C. pneumoniae* or other infectious agents, such as parvovirus B19 or herpes virus. A common pathway between GCA and atherosclerosis has been suggested (10). Atherosclerosis is a multifactorial, multistep disease that involves chronic inflammation and that all the risk factors contribute to pathogenesis by amplifying the underlying inflammatory process. In this regard, infection can stimulate atherogenic processes and there are significant interactions between infection and traditional risk factors. Precisely, anti-human HSP and infectious HSP antibodies have been related to the prevalence of atherosclerosis (11).

cHSP60 shows a great homology with human HSP60 (hHSP60) and *C. Pneumoniae* infection can induce immune reactivity against hHSP60, which may serve as a target for autoimmune reactions (12). This may be especially relevant in GCA, which courses with lesions in smooth muscle cells of medium to large vessels, since these cells express high levels of hHSP60. Both humoral and cellular immune responses against cHSP60 and hHSP60 have been found in atherosclerosis and other cardiovascular diseases (12). Following the parallelism between atherosclerosis and GCA indicated by Regan *et al.* (7), we measured serum levels of hHSP60 and *C. Pneumoniae* antibodies (Abs) in a cohort of GCA patients with active untreated disease.

Competing interests: none declared.

### Material and methods

Seventeen GCA patients with active untreated disease were included in the study. As controls, we employed 23 aged-matched healthy subjects and 39 patients with active untreated PMR, which is a close-related disease to GCA (1). The study was approved by the Ethics Committee at the Hospital Universitario Marqués de Valdecilla and all the patients gave informed consent before obtaining blood samples for the study. Corticosteroid therapy for GCA patients was started with prednisone 40-60 mg daily. PMR patients were treated with prednisone 10 mg daily. After the initial control of the disease, the dose of corticosteroids was progressively reduced according to clinical disease activity. Serum samples were obtained at disease onset and after clinical remission with steroid therapy.

IgG Abs reacting with recombinant hHSP60 (Sigma Chemical Co, St Louis, MO) were quantified by an in-house ELISA. Microtitre plates were coated with 5 µg/ml of recombinant hHSP60 in phosphate buffered saline (PBS) overnight at 4°C. After blocking with PBS-2% bovine serum albumin (BSA), samples were run in triplicate at a 1/500 serum dilution. Sera were incubated for 3 hours at room temperature and after 3 washes, wells were incubated with 100 µl of a goat F(ab')<sub>2</sub> anti-human IgG (Fc)-phosphatase alkaline conjugate (Dako, Glostrup, Denmark) diluted 1:1000 in PBS-2% BSA. After three additional washes, fresh substrate (p-Nitrophenyl Phosphate disodium, Sigma-Aldrich co) was added for 1 hour to each well and absorbances were read at 405 nm. Results were given in mean optical densities (OD) obtained at 405 nm, calculated after subtraction of OD obtained in parallel plates with no coated hHSP60. IgG Abs specific for *C. pneumoniae* were measured with an available ELISA (Thermo Labsystems, Helsinki, Finland) according to the manufacturer's instructions and results were calculated as enzymatic immuno-units with respect to a calibrator.

Statistical analysis of results was performed with SPSS 12.0 Software (SPSS, Chicago, IL, USA). Differences between groups of patients were evaluated

by the Mann-Whitney U-test. Statistical significance of differences in Abs titres at clinical onset and after clinical remission was assessed by the Wilcoxon rank-sum test. *P*-values <0.05 were considered as statistically significant.

### Results

#### *Decrease of anti-chamidial pneumoniae antibodies in GCA and PMR after steroid-induced remission*

The demographic features and vascular risk factors in the study patients are shown in Table I. As shown in Figure 1, patients with GCA had similar serum levels of IgG *C. pneumoniae* Abs,

compared with aged-paired healthy subjects. Although PMR patients had a slight increase in IgG *C. pneumoniae* Abs as compared with GCA patients and healthy subjects, these differences did not reach statistical significance. Remarkable enough was the decrease of IgG *C. pneumoniae* Abs titres after steroid therapy in both GCA (29.1% of reduction, *p*=0.04) and PMR (25.9% of reduction, *p*=0.03) patients (Fig. 1A).

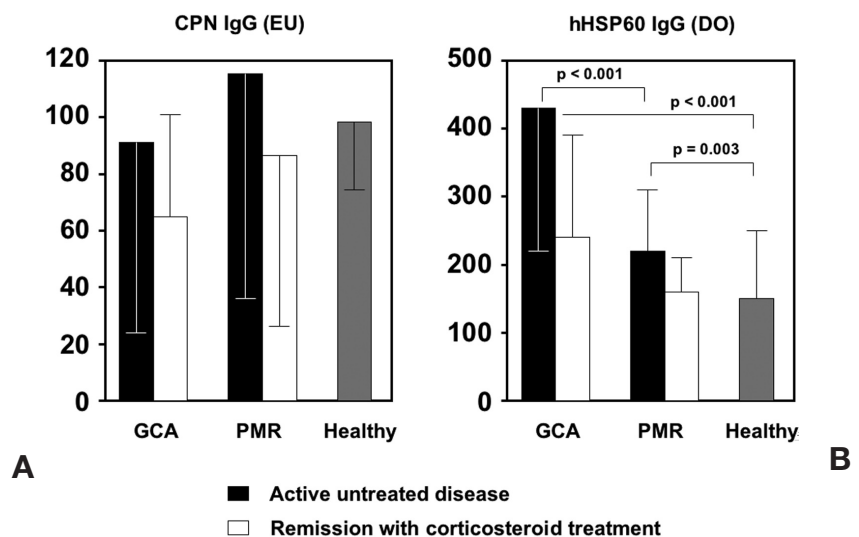
*Anti-human HSP60 antibodies are elevated in GCA patients compared to PMR patients and healthy subjects*  
Since cHSP60 and hHSP60 have a high

**Table I.** Main clinical and laboratory data of giant cell arteritis (GCA) and polymyalgia rheumatica (PMR) patients.

	GCA	PMR	Healthy subjects
Number	17	39	23
Age (years)	73.3 ± 5.6	72.1 ± 7.8	73.2 ± 6.6
Sex (% females)	76	51	60
Hypercholesterolemia (%)	6	26	26
Plasma homocysteine (umol/L)*	13.6 ± 4.3	12.7 ± 3.1	11.4 ± 2.8
Ischaemic cardiopathy (%)	12	13	0
ESR (mm 1 hr)**	78 ± 34	46 ± 33	8 ± 5
CRP (mg/dl)**	7.1 ± 5.3	3.5 ± 3.2	0.3 ± 0.6

ESR: erythrocyte sedimentation rate; CRP: C reactive protein.

\*Plasma homocysteine levels in patients with GCA and PMR were significantly higher than in healthy subjects (*p*<0.05). \*\*Differences for ESR and CRP between GCA and PMR patients were statistically significant (*p*<0.05). Differences between both types of patients and aged-paired healthy subjects were also significant (*p*<0.05).



**Fig. 1.** Serum levels of IgG antibodies to *Chlamydia pneumoniae* (*C. pneumoniae* IgG, **A**) and human heat shock protein (hHSP) 60 IgG, (**B**) in patients with GCA compared to PMR patients and healthy aged-matched subjects. Bars represent mean values and error bars denote standard deviations. *C. pneumoniae* IgG were quantified as enzymatic units (EU) whereas hHSP60 IgG were determined as optical density (OD) at 405. Differences among groups were only significant for human hHSP60 antibodies as indicated in the figure. Changes in each patient group after remission (white bars) with steroid therapy were always significant as compared with onset levels (black bars), as indicated in the text.

homology, we next measured serum levels of IgG hHSP60 Abs (Figure 1B). In contrast with *C. pneumoniae* Abs, IgG hHSP60 Abs were significantly increased in GCA patients in comparison with PMR patients (two-fold) and healthy controls. Nevertheless, IgG hHSP60 Abs were also significantly increased in PMR patients with respect to healthy controls, although slightly. As observed for *C. pneumoniae* Abs, serum IgG hHSP60 Abs decreased significantly almost two-fold with steroid therapy in both groups of patients ( $p=0.04$  and  $p=0.01$  for GCA and PMR, respectively). Finally, despite the homology between cHSP60 and hHSP60, we did not observe any correlation between *C. pneumoniae* Abs and hHSP60 Abs (data not shown).

## Discussion

Although our serological results cannot replace molecular approaches to directly detect *C. pneumoniae* (7, 8), it is important to consider that the encounter with the infectious agent in GCA patients is considered to be previous to the development of the symptomatic disease. Therefore, it is possible that the bacterium is no longer detectable locally and the serology may be the only indication of *C. pneumoniae* involvement. In fact, the search for *C. pneumoniae* genes in atherosclerotic plaques, where its role is better established, has demonstrated only traces of *C. pneumoniae* DNA (13). This may be a possible explanation for the negative results reported, since they only looked out for the presence of the *C. pneumoniae* MOMP gene (7) or *C. pneumoniae* MOMP and 16S rRNA genes (8).

Our data point for an initial immune response against unknown infectious agent(s), which generates immune memory. Later on, due to the high interspecies homology of the HSP ("molecular mimicry"), self-HSP may be recognized by the immune system and produce hHSP60 Abs, as described in other vasculopathies. It is also important to consider that cHSP, together with lypopolysaccharide, are the most immunogenic particles of *C. pneumoniae*, at difference of the MOMP searched by Regan *et al.* (7) and Helweg-Larsen *et*

*al.* (9), which is weakly immunogenic. It is somehow intriguing the increased production of anti-hHSP60 autoantibodies in diseases such as GCA and PMR that are characterized by their potent cellular response with no evidence of a humoral one. In fact, no autoantibody of clinical relevance or hypergammaglobulinemia has been described in these disorders. One possible argument for our finding could be related to the age of GCA and PMR patients. However, it seems to be not age-related since our control subjects were age-paired. In spite of such specificity, we consider that anti-human hHSP60 can not be considered as serological markers of the disease but as reflect of a previous contact with infectious agent(s) with special tropism for cardiovascular tissues. Furthermore, it is possible that the presence of these autoantibodies is an indirect effect of hHSP specific T cells, as has been recently demonstrated in Takayasu arteritis (14). Although we have no evidence for it, it is also possible that we have measured a part of anti-endothelial cell Abs since hHSP60 is an important target for those Abs, especially in vasculitis-associated systemic autoimmune disease (15). A proposed mechanism in the pathophysiology of autoimmune diseases, such as GCA and PMR, is through the induction of pro-inflammatory cytokine production by monocytes via Toll-like receptors (16). Finally, high levels of anti-hHSP60 autoantibodies have been associated with increasing severity of atherosclerosis in patients (17). More importantly, acute *C. pneumoniae* infection with secondary HSP60 humoral response has been involved in the pathophysiology of acute coronary syndromes (18-20). In fact, an advance atherosclerosis has been suggested as a possible susceptible factor to explain the development of these disorders in elderly patients (21). In agreement with it, it is possible that the vascular inflammation that has been demonstrated in GCA might be the inducer of a secondary humoral response against hHSP60 as demonstrated in the present work. However, no correlation was found of ESR and CRP with serum levels of anti-hHSP60 antibodies.

In summary, the protective immune response against *C. pneumoniae* and/or other unknown infectious agent(s) may carry the danger of a crossreactivity with self-HSP, which are expressed in endothelial cells. When other environmental factors participating in the pathogenesis of GCA induce the activation of memory autoreactive B cells and their differentiation to plasma cells, hHSP Abs are produced. Unspecific immunosuppression with steroids would reduce such an activation and would reduce the levels of serum Abs. Our data suggests a role of infectious agents in GCA and might support the hypothesis that *C. pneumoniae* might be only one more among the possible players in this disease. Nonetheless, other triggering factors, such as vascular damage, itself cannot be discarded.

## Acknowledgments

We are especially grateful to Iñaki Beares, Ainhoa Bolívar and María San Martín for their helpful technical assistance.

## References

1. SALVARANI C, CANTINI F, BOIARDI L: Polymyalgia rheumatica and giant-cell arteritis. *N Engl J Med.* 2002; 347: 261-71.
2. DASGUPTA B, HASSAN N: British Society for Rheumatology Guidelines Group. Giant cell arteritis: recent advances and guidelines for management. *Clin Exp Rheumatol* 2007; 25 (Suppl. 44): S62-5.
3. WARRINGTON KJ, MATTESON EL: Management guidelines and outcome measures in giant cells arteritis (GCA). *Clin Exp Rheumatol* 2007; 25 (Suppl. 47): S137-41.
4. WEYAND CM, GORONZY JJ: Giant-cell arteritis and polymyalgia rheumatica. *Ann Intern Med* 2003; 139: 505-15.
5. RIMENTI G, BLASI F, COSENTINI R *et al.*: Temporal arteritis associated with *Chlamydia pneumoniae* DNA detected in an artery specimen. *J Rheumatol* 2000; 27: 2718-20.
6. WAGNER AD, GERARD HC, FRESEMANN T *et al.*: Detection of *Chlamydia pneumoniae* in giant cell vasculitis and correlation with the topographic arrangement of tissue-infiltrating dendritic cells. *Arthritis Rheum* 2000; 43: 1543-51.
7. REGAN MJ, WOOD BJ, HSIEH YH *et al.*: Temporal arteritis and *Chlamydia pneumoniae*. Failure to detect the organism by polymerase chain reaction in ninety cases and ninety controls. *Arthritis Rheum* 2002; 46: 1056-60.
8. HELWEG-LARSEN J, TARP B, OBEL N *et al.*: No evidence of parvovirus B19, *Chlamydia pneumoniae* or human herpes virus infection in temporal artery biopsies in patients with giant cell arteritis. *Rheumatology (Oxford)* 2002; 41: 445-9.

9. SAIKKU P: *Chlamydia pneumoniae* in atherosclerosis. *J Intern Med* 2000; 247: 391-6.
10. DUHAUT P, PINEDE L, DEMOLONBE-RAGUE S *et al.*: Giant cell arteritis and cardiovascular risk factors. A multicenter, prospective case-control study. *Arthritis Rheum* 1998; 41: 1960-5.
11. AYADA K, YOKOTA K, KOBAYASHI K *et al.*: Chronic infections and atherosclerosis. *Ann N Y Acad Sci* 2007; 1108: 594-602.
12. WICK G, PERSCHINKA H, MILLONIG G: Atherosclerosis as an autoimmune disease: an update. *Trends Immunol* 2001; 22: 665-9.
13. VALASSINA M, MIGLIORINI L, SANSONI A *et al.*: Search for *Chlamydia pneumoniae* genes and their expression in atherosclerotic plaques of carotid arteries. *J Med Microbiol* 2001; 50: 228-32.
14. CHAUHAN SK, SINGH M, NITYANAND S: Reactivity of gamma/delta T cells to human 60-kd heat-shock protein and their cytotoxicity to aortic endothelial cells in Takayasu arteritis. *Arthritis Rheum* 2007; 56: 2798-802.
15. JAMIN C, DUGUÉ C, ALARD JE *et al.*: Induction of endothelial cell apoptosis by the finding of anti-endothelial cell antibodies to Hsp60 in vasculitis-associated systemic autoimmune diseases. *Arthritis Rheum* 2005; 52: 4028-38.
16. YOKOTA S, MINOTA S, FUJII N: Anti-HSP auto-antibodies enhance HSP-induced pro-inflammatory cytokine production in human monocytic cells via Toll-like receptors. *Int Immunol* 2006; 18: 573-80.
17. MANDAL K, FOTEINOS G, JAHANGIRI M *et al.*: Role of antiheat shock protein 60 autoantibodies in atherosclerosis. *Lupus* 2005; 14: 742-6.
18. BIASUCCI LM, LIUZZO G, CIERVO A *et al.*: Antibody response to chlamydial heat shock protein 60 is strongly associated with acute coronary syndromes. *Circulation* 2003; 107: 3015-7.
19. HELTAI K, KIS Z, BURIAN K *et al.*: Elevated antibody levels against *Chlamydia pneumoniae*, human HSP60 and mycobacterial HSP65 are independent risk factors in myocardial infarction and ischaemic heart disease. *Atherosclerosis* 2004; 173: 339-46.
20. HOSHIDA S, NISHINO M, TANOUCHI J *et al.*: Acute *Chlamydia pneumoniae* infection with heat-shock-protein-60-related response in patients with acute coronary syndrome. *Atherosclerosis* 2005; 183: 109-12.
21. MARTINEZ-TABOADA VM, BARTOLOME MJ, FERNANDEZ-GONZALEZ MD *et al.*: Homocysteine levels in polymyalgia rheumatica and giant cell arteritis: influence of corticosteroid therapy. *Rheumatology (Oxford)* 2003; 42: 1055-61.