Elevated levels of serum antibodies against Saccharomyces cerevisiae mannan in patients with systemic lupus erythematosus

H Dai, Z Li, Y Zhang, P Lv and XM Gao

Lupus 2009 18: 1087
DOI: 10.1177/0961203309105131

Lupus (2009) 18, 1087–1090
http://lup.sagepub.com

CONCISE REPORT

Elevated levels of serum antibodies against *Saccharomyces cerevisiae* mannan in patients with systemic lupus erythematosus

H Dai[1,2], Z Li[3], Y Zhang[1], P Lv[1] and XM Gao[1,2]
[1]Department of Immunology, Peking University Health Science Center, Peking University, Beijing, China; [2]Key Laboratory for Immunology, Ministry for Public Health, Beijing, China; and [3]Department of Rheumatology, People's Hospital, Peking University, Beijing, China

This study was undertaken to investigate whether levels of anti-*Saccharomyces cerevisiae* mannan antibodies (ASCMAs), a serological marker for Crohn's disease, seronegative spondyloarthritis and Behcet's disease, also correlate with systemic lupus erythematosus (SLE) in humans. Serum samples from healthy volunteers ($n = 152$) and patients with SLE ($n = 40$) were compared for ASCMA-IgA, -IgG and -IgM levels using enzyme linked immunosorbent assays. ASCMA-IgG, but not IgM and IgA, prevalence was significantly raised in active SLE patients (57.5%) compared with healthy controls (8.5%). ASCMA-IgG levels in SLE patients during remission were relatively lower, indicating a possible correlation with disease activity. These results differ from a previous study, which did not detect a difference between ASCMA levels in SLE patients and healthy control. It remains to be evaluated whether elevated ASCMA-levels are common to all rheumatic disorders. *Lupus* (2009) **18,** 1087–1090.

Key words: α-1,3-mannan; autoantibody; autoimmune diseases; SLE

Introduction

Anti-*Saccharomyces cerevisiae* mannan antibodies (ASCMAs) are considered as a serological marker for Crohn's disease (CD), a chronic inflammatory disorder of the intestine.[1–3] It has also been shown that ASCMAs have predictive value for inflammatory bowl disease.[4] Additionally, elevated levels of ASCMA have also been found in patients with Behcet's disease, autoimmune hepatitis and also seronegative spondyloarthritis.[5–8] Systemic lupus erythematosus (SLE) is characterised by the production of polyreactive autoantibodies against a wide variety of antigens, including glycan, lipid, double-stranded DNA and other nuclear antigens.[2,9,10] It is possible that a higher prevalence of ASCMA might also be seen in patients with SLE. However, a previous study found ASCMA in only 1 of 10 SLE patients.[5] The present study was undertaken to reevaluate whether ASCMAs are elevated in patients with SLE.

Correspondence to: Dr XM Gao, Department of Immunology, Peking University Health Science Center, 38 Xueyuan Road, Beijing 100191, China. Email: xmgao@bjmu.edu.cn
Received 5 December 2008; accepted 2 March 2009

Materials and methods

Human subjects and blood samples

Serum samples were collected from 152 healthy volunteers (Table 1). Serum samples were collected from 40 hospitalised patients, attending the Department of Rheumatology and Immunology, Peking University People's Hospital, Beijing between 2006 and 2007, with active SLE fulfilling the American Rheumatism Association criteria for diagnosis of SLE.[9] All SLE patients donated blood samples while their disease was in flare, five of them also donated while in a convalescence phase. The blood samples were processed within 18 h of collection, and the sera stored at −80 °C until use. Average British Isles Lupus Assessment Group (BILAG) score of these SLE patients was 13.4 ± 5.2 (range 8.3–18.1). Prednisone was administered for steroid treatment (15–60 mg q.d., median dose 34.8 ± 17.3 mg). For disease-modifying antirheumatic drug (DMARD) treatment, hydroxychloroquine (0.2 g b.i.d.) and cyclophosphamide (50 mg b.i.d.) were given. This study has been reviewed and approved by the ethics committee of Peking University Health Science Center.

10.1177/0961203309105131

Table 1 Subject groups and their ASCMA prevalence[a]

Subject groups	*Healthy subjects*	*SLE patients*
Number of subjects	152	40
Mean age, year (range)	30.7 (20–50)	31.7 (17–51)
Female/male	85/67	36/4
Duration of disease, year (range)	—	6.01 (1–12)
ASCMA-IgG	8.5% (13/152)	57.5% (23/40)[b] $P < 0.0001$[c]
ASCMA-IgA	5.3% (8/152)	7.5% (3/40) $P = 0.469$
ASCMA-IgM	5.3% (8/152)	0% (0/40) $P = 0.060$

Abbreviations: ASCMA: anti-Saccharomyces cerevisiae mannan antibodies; SLE: systemic lupus erythematosus.
[a]A summary of the data showing the prevalence (percent positive) of ASCMA in healthy subjects and patients with SLE. The samples with binding indices above the cutoff values were scored as positive.
[b]The percentages (number of positive/total number in group) of subjects positive for relevant ASCMA Abs are shown.
[c]Fisher's exact tests were performed by comparing ASCMA prevalence of patient groups with healthy adults, and the *P* values are shown.

Mannan-based ELISA

Mannan of *Saccharomyces cerevisiae* (M7504) and D-Mannose (M6020) were purchased from Sigma (Saint Louis, MO, USA). Flat bottom 96-well microtitre plates (Corning costar) were coated with 50 μg/mL mannan in 0.1 M phosphate-buffered saline (PBS) (pH 9.6) at 4 °C overnight. The plates were washed with 0.05% Tween 20 (Sigma) in PBS between each stage. Each plate was blocked with 10% foetal calf serum (FCS) in PBS for 2 h at 37 °C. Serum samples were diluted 1:200 in 2% FCS in PBS and incubated in the ELISA wells for 2 h at 37 °C. Detection of IgM, IgG or IgA was done using goat anti-human IgM, IgG or IgA coupled to Horseradish peroxidase (Southern Biotechnology Inc, Birmingham, Alabama, USA) diluted 1:4000 in PBS-Tween and incubated for 1 h at 37 °C. The reaction was developed with 100 μL of O-phenylenediamine (Sigma) for 5 min and stopped with 100 μL 3 M H_2SO_4. Optical density (OD) was measured at 492 nm using an ELISA spectrophotometer. The OD reading of the control sample on each plate, controlled at 0.2–0.3 throughout the study, was used to calculate the binding index (BI) of the specimen: BI = OD (sample)/OD (control).

Statistical analysis

All serum samples were assayed for ASCMA IgG, IgA and IgM at least three times with consistent results. Statistical analysis was performed using SPSS software (Chicago, IL, USA). Significance was defined as a *P* value <0.05. A receiver operating characteristics (ROC) curve was generated by plotting sensitivity (*y*-axis) against 1-specificity (*x*-axis).

Results

Elevated ASCMA-IgG levels in SLE

Serum samples from 152 healthy adults were screened for ASCMA-IgG using *S. cerevisiae* mannan-based ELISA, in which 13 of the 152 samples (8.5%) were high responders compared with the rest of the group (Figure 1A). These samples were also screened for ASCMA-IgA and ASCMA-IgM, very few strong responders were found (Figure 1C,D). An equal proportion mixture of these sera was prepared and used as negative control in subsequent experiments.

As also shown in Figure 1A and Table 1, the majority of serum samples from SLE patients (57.5%) were positive for ASCMA-IgG, significantly more than that observed in healthy subjects (two-sided Fisher's exact test, $P < 0.0001$). These results remained significant when ASCMA IgG levels were corrected for total IgG levels ($P < 0.0001$). Further analysis of serially diluted serum samples from representative SLE patients (SLE-2 and SLE-25) confirmed the screening results (Figure 1B). We could not demonstrate that ASCMA-IgM or IgA levels in

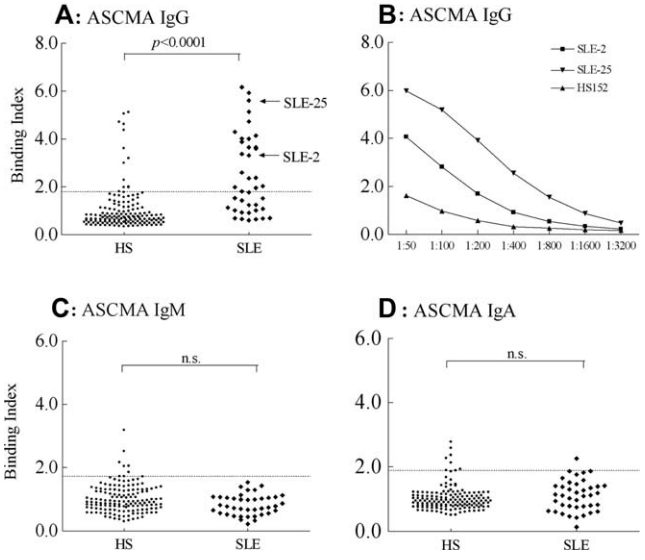

Figure 1 Comparison of ASCMA levels in SLE patients and healthy subjects. ASCMA-IgG (A), IgM (C) and IgA (D) in sera from healthy adult human subjects (HS: healthy subjects, $n = 152$) and SLE patients ($n = 40$) were individually assayed. The OD at 492 nm was measured, and the reading for the negative control serum controlled at 0.2–0.28. The results were calculated as binding indices (BI), and the mean BI of the three wells are shown. Horizontal lines represent cutoff values calculated using the ROC curves. Mann–Whitney U test was performed comparing SLE patient group with the healthy controls, and the *P* values are given in the figure. Serum samples from representative SLE patients (*SLE-2, SLE-25*) were serially diluted and compared with control serum (*HS152*) in parallel experiments (B).

patients with SLE were significantly higher than subjects (Figure 1C,D).

ASCMA-IgG correlation with SLE activity

Additional serum samples were collected from five SLE patients when their disease was in remission and compared with their active phase sera. In all five cases, the latter contained higher levels of ASCMA-IgG than the former (Figure 2A), indicating a positive correlation between ASCMA-IgG levels and SLE disease activity ($P < 0.05$, Wilcoxon signed-rank test).

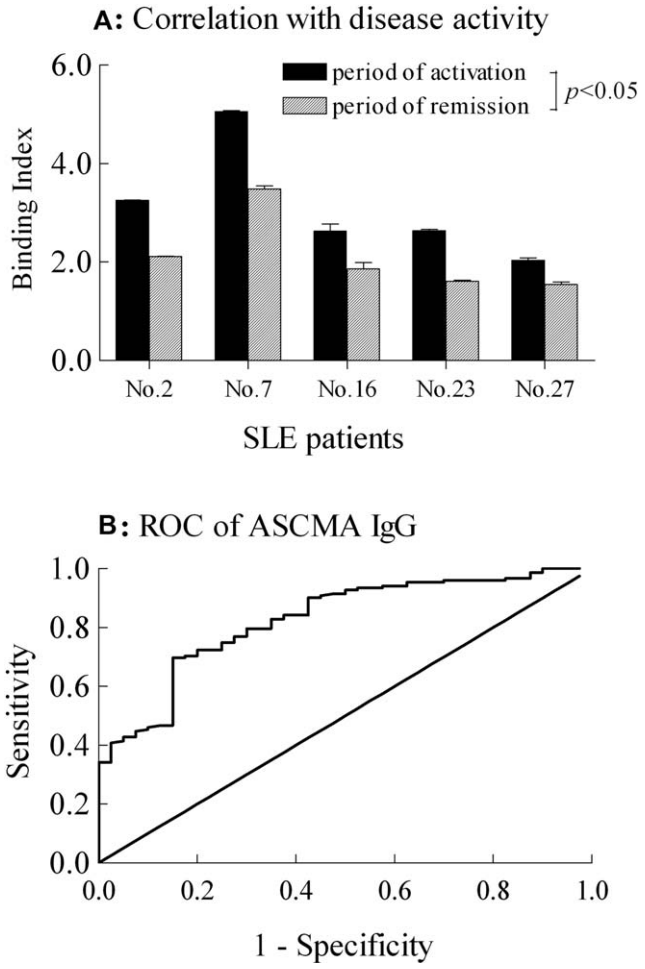

Figure 2 Correlation between ASCMA-IgG levels and SLE activity. Two serum samples were collected from each one of five SLE patients when their disease was in flare and in remission. The remission phase sera were compared with active phase sera for ASCMA IgG levels using mannan-based ELISA, and the results are expressed as mean binding indices (A). Wilcoxon signed-rank test was performed for paired comparison, and the *P* value shown in the figure. ROC curves for ASCMA-IgG were obtained by plotting sensitivity for detecting SLE against specificity. Healthy subjects were used as the control group (B). Area under curve = 0.826.

ROC curve analysis for ASCMA-IgG

ROC curves were plotted for ASCMA-IgG to evaluate sensitivity and specificity of our mannan-based ELISA system in diagnosing SLE. We also performed ROC curve analysis to determine the optimal cutoff value for ASCMA in SLE (Figure 2B). With a cutoff value at 1.82 the sensitivity for detecting SLE is 57.5%, whereas the specificity is 89.2%.

Discussion

Our present results show that ASCMA-IgG levels are significantly elevated in patients with SLE and correlate with disease activity. Krause and colleagues did not find significant difference between SLE patients and control subjects in levels of ASCMA.[5] There are several possible, but not necessarily mutually exclusive, explanations for these discrepancies. First, the relatively small sample size in these studies may be partially responsible for the inconsistency. Further studies on larger populations of various ethnic backgrounds are necessary to draw more definitive conclusions. Additionally, although the patients included in these studies fulfilled the same diagnostic criteria, the different genetic background may influence their ability to make ASCMA. This is supported by the fact that elevated ASCMA have been reported in 20% healthy relatives of patients with CD compared with 8% in randomly selected healthy controls.[11,12] In our study, all 40 SLE patients belong to Chinese Han ethnic group, whereas studies carried out in other countries would have recruited patients of different ethnic background. Finally, the assays used to measure ASCMA are not identical. We established our own ELISA systems, whereas the other groups used commercially available kits. We diluted all serum samples 1/200 for screening tests, whereas the other studies used different dilutions.

The mechanisms for elevated production of ASCMA in patients with SLE or other autoimmune diseases remain unclear. It is possible that ASCMA are autoantibodies produced as a consequence of an autoimmune response in the absence of an infection but which cross-react with *S. cerevisiae* mannan. Mannan is part of the glycosylation moieties of N-linked glycoproteins in humans and other animals, which could lead to mannan-specific tolerance on one hand and production of anti-mannan autoantibodies on the other. In consistence with this notion, previous studies have shown that ASCMA cross-reacted with β2-glycoprotien-I.[13] Another possibility is that, as a result of reduced defense ability of patients with autoimmune diseases, subclinical infection with

S. cerevisiae or other microorganisms carrying mannan epitopes occurs more easily and therefore leads to increased production of ASCMA. Saiki, *et al.* reported that recombinant yeast expressing paraneoplastic cerebellar degeneration-associated antigen could effectively induce production of specific autoantibodies in mice.[14] Persistence of an infectious agent has been implicated in various forms of rheumatic disorders.[15] We suggest that the balance between self-tolerance (immunoregulation) and environmental stimuli (microbial infection) determines the levels of ASCMA in human subjects. Under constant stimulation by mannan-containing antigens of microorganisms, the lack of ASCMA in the majority of healthy human subjects is the result of successful self-tolerance maintenance, whereas production of such Abs in patients is a consequence of natural tolerance breakdown due to immunodysregulation.

Another key question is whether ASCMA have any pathophysiological role to play in the pathogenesis of autoimmune diseases. It seems that at least they alone are unlikely to cause any pathological damage, since a small percentage (8.5%) of healthy subjects have ASCMA at titres similar to that in SLE patients (Figure 1). A prospective study on such individuals may evaluate whether this would have any predictive value for possible autoimmune disorders in their later lives.

Acknowledgements

This study was supported by grants from the National Key Basic Research Programs (2007CB512406), '863' Program (2006AA02Z495) and also Natural Science Foundation of China (30371303; 31890142).

References

1 Damoiseaux, JG, Bouten, B, Linders, AM, *et al*. Diagnostic value of anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic antibodies for inflammatory bowel disease: high prevalence in patients with celiac disease. *J Clin Immunol* 2002; **22**: 281–288.
2 Dotan, N, Altstock, RT, Schwarz, M, Dukler, A. Anti-glycan antibodies as biomarkers for diagnosis and prognosis. *Lupus* 2006; **15**: 442–450.
3 Dotan, I, Fishman, S, Dgani, Y, *et al*. Antibodies against laminaribioside and chitobioside are novel serologic markers in Crohn's disease. *Gastroenterology* 2006; **131**: 366–378.
4 Israeli, E, Grotto, I, Gilburd, B, *et al*. Anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic antibodies as predictors of inflammatory bowel disease. *Gut* 2005; **54**: 1232–1236.
5 Krause, I, Monselise, Y, Milo, G, Weinberger, A. Anti-Saccharomyces cerevisiae antibodies–a novel serologic marker for Behcet's disease. *Clin Exp Rheumatol* 2002; **20**: S21–S24.
6 Muratori, P, Muratori, L, Guidi, M, *et al*. Anti-Saccharomyces cerevisiae antibodies (ASCA) and autoimmune liver diseases. *Clin Exp Immunol* 2003; **132**: 473–476.
7 Hoffman, IE, Demetter, P, Peeters, M, *et al*. Anti-Saccharomyces cerevisiae IgA antibodies are raised in ankylosing spondylitis and undifferentiated spondyloarthropathy. *Ann Rheum Dis* 2003; **62**: 455–459.
8 Riente, L, Chimenti, D, Pratesi, F, *et al*. Antibodies to tissue transglutaminase and Saccharomyces cerevisiae in ankylosing spondylitis and psoriatic arthritis. *J Rheumatol* 2004; **31**: 920–924.
9 Tan, EM, Cohen, AS, Fries, JF, *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; **25**: 1271–1277.
10 Hahn, BH, McMahon, M. Atherosclerosis and systemic lupus erythematosus: the role of altered lipids and of autoantibodies. *Lupus* 2008; **17**: 368–370.
11 Veys, EM, Mielants, H. Enteropathic arthropathies. In: Klippel, J, Dieppe, PA, (ed), Rheumatology. London: Mosby International; 1998. p. 1–8.
12 Bourne, JT, Kumar, P, Huskisson, EC, Mageed, R, Unsworth, DJ, Wojtulewski, JA. Arthritis and coeliac disease. *Ann Rheum Dis* 1985; **44**: 592–598.
13 Krause, I, Blank, M, Cervera, R, *et al*. Cross-reactive epitopes on beta2-glycoprotein-I and Saccharomyces cerevisiae in patients with the antiphospholipid syndrome. *Ann N Y Acad Sci* 2007; **1108**: 481–488.
14 Saiki, M, Sakai, K, Saiki, S, Kitagawa, Y, Nakanishi, M, Hirose, G. Induction of humoral responses specific for paraneoplastic cerebellar degeneration-associated antigen by whole recombinant yeast immunization. *J Autoimmun* 2005; **24**: 203–208.
15 Klein, J, Horejsi, V. Defense reactions mediated by macrophages. In: Klein, J, (ed), Immunology. 2nd ed. Oxford: Blackwell Science; 1997. p. 442–447.