BRIEF PAPER

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Antibodies to human cytomegalovirus protein UL83 in systemic sclerosis

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ABSTRACT

Objective. *To determine whether elevated levels of antibodies to HCMV protein UL83 were present in patients with SSc and if their prevalence was associated with major SSc-associated autoantibodies.*

Methods. *The study population consisted of 253 Caucasian subjects (110 SSc patients and 143 controls). IgG antibodies to UL83 were measured by an enzyme-linked immunosorbent assay (ELISA). Antibodies to centromere and RNA polymerase (RNAP) were determined by indirect immunofluorescence and immnoprecipitation methods, respectively.*

Results. *The mean level of anti-UL83 antibodies in the sera of SSc patients as a whole was significantly higher than that in control subjects (14.75 vs 10.6 units/μl, p = 0.002). Both subgroups of patients contributed to this variation: compared to controls, anti-UL83 antibody levels were higher in diffuse (16.32 vs 10.6 units/μl, p = 0.012) as well as in those with the limited form of the disease (13.95 vs 10.6 units/μl, p = 0.015). Anti-UL83 antibodies were not associated with major SSc-associated autoantibodies.*

Conclusion. *Humoral immunity to HCMV protein UL83 may be relevant to the etiopathogenesis of scleroderma.*

Introduction

Systemic sclerosis is a systemic connective tissue disease characterized by extensive fibrosis, arterial fibrointimal proliferation, and autoantibody production (1). Although the etiology of SSc is not known, there is increasing evidence for potential environmental triggers, possibly from pathogenic organisms, in the induction of the disease in genetically susceptible individuals (2). The downstream effect of HCMV infection on immune, vascular, and tissue repair mechanisms, as well as its propensity to infect endothelial cells and fibroblasts, constitute associative evidence that led to the hypothesis that HCMV is an accelerating factor for the pathologies associated with SSc (3). Several observations, such as elevated levels of antibodies to HCMV or its protein components in SSc patients (4-6), sequence similarity between HCMV protein UL70 and human topoisomerase I (7), sequence similarity between HCMV protein UL94 and an SSc peptide (5), and appearance of SSc symptoms after infection with HCMV (8), at least indirectly, support this hypothesis.

Molecular mimicry - immune response against antigens shared by the host and a virus - has been postulated to be a possible mechanism by which HCMV could initiate autoimmunity in SSc (9). Alternatively, possible association of nuclear proteins with HCMV antigens may make these proteins immunogenic. As HCMV protein UL83 is a nuclear protein and one of the major targets of host immune defense mechanisms against HCMV infection (10), we sought to determine whether elevated levels of antibodies to HCMV protein UL83 were present in patients with SSc. In addition, we wished to determine whether these antibodies were associated with the prevalence of SSc-specific anti-topoisomerase I (topo I), anti-centromere (ACA) and anti-RNA polymerase I/III (RNAP) antibodies, which would be expected if they crossreact with these autoantigens or if the immune response is directed against viral-self protein complexes.

Materials and methods

Subjects

A total of 110 SSc patients, presenting at the Division of Rheumatology, Medical University of South Carolina, consented to participate in the study. All SSc patients fulfilled the American College of Rheumatology Criteria for systemic sclerosis (11), were of Caucasian origin, and the majority (67%) of patients had the limited form of SSc. Controls (143) consisted of patients with osteoarthritis, fibromyalgia, gout, and localized musculo-skeletal pain syndromes, attending the same clinic as the patients. Controls with conditions associated with autoimmune or connective tissue diseases were excluded. All subjects were unrelated. Blood was drawn from patients and controls after informed consent.

Methods

ELISA for the determination of anti-UL83 antibodies. IgG antibodies to UL83 were determined by an ELISA, as described previously (6), with minor modifications. UL83 protein was purchased from Biodesign International (Saco, ME). Polystyrene microtiter plate wells were coated with UL83 (10g/μl) in carbonate-bicarbonate buffer (pH.9.6) and incubated for 1 hr at 37ºC. The wells were washed 5 times with phosphate buffered saline containing 0.05% Tween-20 (PBS-T) and blocked with serum diluent buffer (PBS-T with 1% BSA) for 30 min at 37ºC. Wells were further washed and then incubated with suitably diluted sera (1:200 or more) in serum diluent buffer for 1 hr at 37ºC. Again, wells were washed and then incubated with anti-human IgG peroxidase conjugate (1:2000 in serum diluent buffer) for 30 min at 37ºC. Bound antibodies were measured by incubation with peroxidase substrate, H_2O_2, and TMB as chromagenic substrate. The absorbance was measured at 450 nm. Variations in the absorbance values between experiments were normalized by the use of two serum samples, as standards. Results are expressed as ELISA units/μl of serum, after multiplying the absorbance values with the dilution factor.

Determination of SSc-associated autoantibodies. ACA and anti-RNAP antibodies were determined by indirect immunofluorescence and immunoprecipitation methods, respectively. Double immunodiffusion and protein immunoprecipitation assays were used for measuring antibodies to topo I, as described previously (12). Levels of antibodies to HCMV UL94 were determined by ELISA using a 12 amino acid synthetic peptide as antigen, as described previously (6).

Statistical analysis

Since anti-UL83 antibody levels were not normally distributed (Kolomogorov-Smirnov test, $p < 0.0001$), the non-parametric Mann-Whitney (two-sided) test was used to compare the antibody levels in various groups, using SPSS software (SPSS 12 for Windows). Association between the levels of anti-UL83 and anti-UL94 antibodies was determined by non-parametric Spearman's rank correlation analysis, using SAS software. Statistical significance was defined as $p < 0.05$.

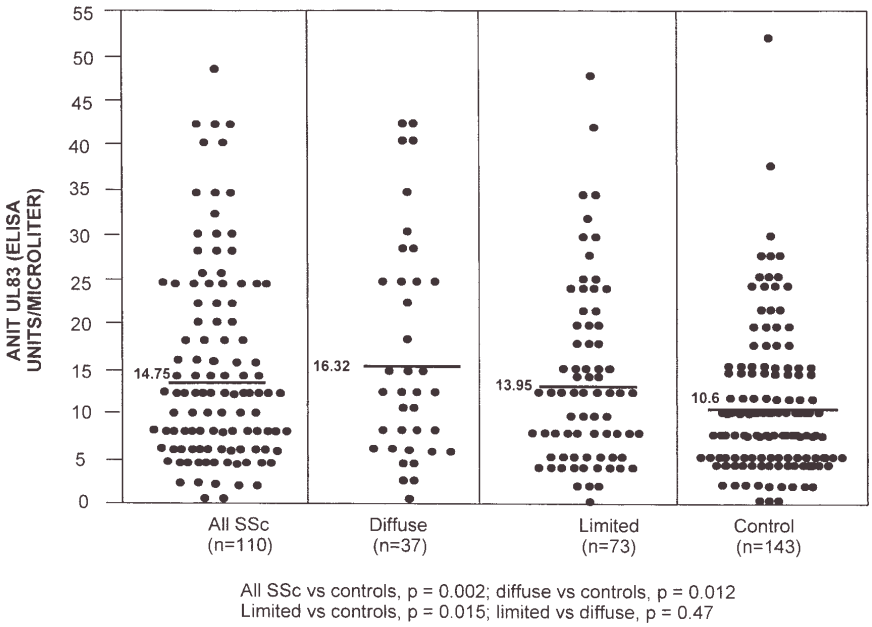

Fig. 1. Binding of IgG antibodies present in the sera of SSc patients (all SSc, diffuse and limited) and controls to HCMV UL83 protein measured by ELISA. Absorbance values obtained are converted to ELISA units after multiplying with the dilution factor. Results shown are the mean values obtained from three independent experiments. Bar line shows the mean value.

Results

The mean levels of anti-UL83 antibodies in SSc patients and controls are given in Figure 1. Antibody levels in SSc patients as a whole were significantly different from that in controls (14.75 vs 10.6 units/μl, $p = 0.002$). Subgroup analysis showed that anti-UL83 antibody levels were significantly higher both in diffuse (16.32 vs 10.6 units/μl, $p = 0.012$) and in limited (13.95 vs 10.6 units/μl, $p = 0.015$) forms of the disease, compared to controls, but no significant differences in antibody levels were found between the two forms of the disease ($p = 0.47$).

There was significant correlation between the levels of anti-UL83 and previously determined anti-UL94 antibodies (6) among SSc patients ($r_s = 0.004$), but not in controls ($r_s = 0.45$). The mean levels of anti-UL83 antibodies in SSc patients were not associated with the prevalence of ACA, anti-topo I or anti-RNAP antibodies (data not shown).

Discussion

The results presented here show that scleroderma patients have elevated levels of antibodies to HCMV protein UL83, and that patients in both disease subgroups contribute to these humoral immune responses. The increased levels of anti-UL83 antibodies in SSc patients were not significantly associated with ACA, anti-topo I or anti-RNAP antibodies. This would suggest that involvement of HCMV in SSc is not through molecular mimicry between UL83 and the self-epitopes recognized by these SSc-associated autoantibodies. However, it is possible that anti-UL83 antibodies crossreact with particular subunits - rather than the entire molecule - of these proteins. Such intramolecular heterogeneity has been reported for anti-RNAP antibodies (13). Furthermore, HCMV may use its other proteins and strategies to dysregulate the autoimmune responses in SSc. We found a strong correlation between the levels of anti-UL83 and previously determined anti-UL94 antibodies in scleroderma patients, but not in controls. This again suggests the possibility of intramolecular heterogeneity in humoral responses to UL83 and UL94 proteins in that the epitopes recognized

by the SSc patients might be different from those recognized by the control subjects. Such heterogeneity, however, is less likely for the UL94 antigen, as it consisted of only 12 amino-acid residues (6), but is possible for the UL83 antigen employed in this study, which consisted of the whole UL83 protein.

These differences in the nature of the two epitopes might have contributed to the observed differences in humoral immunity to these antigens in SSc patients. Anti-UL94 responses were elevated primarily in patients with the diffuse form of the disease (6). In contrast, diffuse patients as well as those with the limited form of the disease contributed to the anti-UL83 responses. The whole UL83 protein - with a larger constellation of antigenic sites than those possessed by the UL94 synthetic peptide of 12 amino-acid residues - is likely to be immunogenic in a larger and more diverse group of individuals.

Longitudinal studies are needed to evaluate whether antibodies to HMCV proteins correlate with disease severity and activity, and whether they antedate particular clinical symptoms in SSc patients, as anti-topo I and anti-RNAP antibodies appear to do (14, 15). Similar studies involving other infectious agents suspected in the etiology of SSc are warranted (16).

HCMV is widespread in the human population, but not all individuals are seropositive, suggesting involvement of host genetic factors in immunity to this pathogen (17). We have shown that in SSc patients GM (genetic markers of IgG heavy chains) genotypes are significantly associated with the occurrence of anti-HCMV antibodies (18). It would be interesting to determine whether the prevalence of anti-UL83 antibodies is also associated with GM allotypes, and whether the GM alleles implicated are the same as those associated with immune responsiveness to the whole virus (18). To our knowledge, this is the first report showing elevated levels of antibodies to HCMV protein UL83 in patients with scleroderma.

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