ABSTRACT

Objective. To determine the role of GM and KM genes — genetic markers of immunoglobulin γ and κ chains, respectively — in humoral immunity to human cytomegalovirus (HCMV) in patients with systemic sclerosis (SSc; scleroderma).

Methods. A total of 137 Caucasian patients with SSc and 145 ethnically matched controls were genotyped for GM f/z, z/17, n+/23+, n-/23-, KM 1, and KM 3 alleles by polymerase chain reaction-restriction fragment length polymorphism and direct DNA sequencing methods. IgG antibodies to HCMV were measured by an enzyme-linked immunosorbent assay.

Results. In SSc patients, GM f,z genotypes were strongly associated with the occurrence of anti-HCMV IgG antibodies. The frequency of the GM f homozygotes was lower (42.2 vs 62.2%; p = 0.02; OR = 0.4) and the frequency of the GM f,z heterozygotes was higher (51.1 vs 26.7%; p = 0.006; OR = 2.8) in SSc patients with IgG antibodies to HCMV than in subjects who lacked these antibodies. This association was not observed in the control group. KM and GM n genotypes were not significantly associated with the prevalence of these antibodies.

Conclusion. GM f,z alleles or alleles in linkage disequilibrium with them influence the generation of IgG antibodies to HCMV in patients with SSc.

Introduction

Discussions with Carwile LeRoy over the years on wide-ranging topics in science have been inspirational to me in all my scientific endeavors. In particular, his fervent commentaries on the pathobiology of the cytomegalovirus ignited my interest to investigate the role of various immunoglobulin and cytokine genes in immunity to this member of the herpes family of viruses. By reading recent papers on molecular mimicry and immune surveillance, I had become fascinated by the sophisticated strategies this virus employs to subvert the immune system, but I had not thought about its possible role in the etiology of systemic sclerosis until Carwile showed me how HCMV could act as a unifying agent for the three apparently disparate pathologies of the sclerodermatous process — immune, vascular, and fibrotic. He invited me to co-author a hypothesis paper (1), which proposed that HCMV, which is able to subvert the immune system for its own purposes, has the propensity to infect endothelial cells and can upregulate fibrogenic cytokines, could serve as an accelerating factor in the observed abnormalities of SSc. Although a direct link between HCMV infection and SSc is still lacking, strong evidence in favor of this proposal has been presented by Lunardi et al. (2). These authors have shown that antibodies against HCMV in SSc patients cause the apoptosis of endothelial cells, considered the initial event in SSc pathogenesis.

In this investigation, I sought to determine whether immunoglobulin (Ig) GM and KM genes, which have been implicated in the production of SSc-associated anticientromere and anti-fibrillin-1 antibodies (3, 4), also contribute to the occurrence of anti-HCMV antibodies in scleroderma patients.

Materials and methods

Study subjects

A total of 137 Caucasian SSc patients presenting at the Division of Rheumatology, Medical University of South Carolina, consented to participate in the study. A group of 145 ethnically matched control subjects was recruited consisting of patients with osteoarthritis, fibromyalgia, gout or regional musculoskeletal pain syndromes attending the same clinic as the patients. Controls with conditions associated with autoimmune or connective tissue diseases.
were excluded. All SSc patients fulfilled the American College of Rheumatology criteria for systemic sclerosis (5). All subjects were unrelated. Blood was drawn from patients and controls after informed consent was obtained.

**GM and KM genotyping**

DNA samples were typed for GM n+/23+, n-/23-, KM 1, and KM 3 alleles by previously described polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods (6,7). For the determination of GM f/z, z/17 alleles, the DNA segment encoding the CH1 region of γ1 chain was amplified by PCR according to Balbin et al. (8) and the purified double-stranded PCR product was subjected to an automatic DNA sequencing on an ABI PRISM 377. (For technical reasons, two patients and four controls could not be genotyped for GM f/z determinants, causing a slight variation in the sample sizes in Tables I-III.)

**Anti-HCMV IgG antibody determination**

Serum IgG HCMV antibodies were measured in all subjects using the CMV IgG ELISA kit from Wampole Laboratories (Cranbury, NJ). The international standardized ratio (ISR), a semi-quantitative measure of the relative level of antibody, was calculated for all subjects, following the manufacturer’s instructions.

**Statistical analyses**

The prevalence of IgG antibodies to HCMV and the distribution of genotype frequencies in patients and controls were analyzed using Pearson’s $\chi^2$ test. Statistical significance was defined as $p < 0.05$. Odds ratio (OR) was calculated to measure the strength of the association observed. Calculations were made using the Internet program http://home.clara.net/sisa/two2hlp.htm.

**Results**

The distribution of GM f/z genotypes in scleroderma patients and controls with or without IgG antibodies to HCMV is given in Table I. In SSc patients, these genotypes were significantly associated with the occurrence of anti-HCMV antibodies. The frequency of the GM f homozygotes was lower (42.2 vs 62.2%; $p = 0.02$; OR $= 0.4$) and the frequency of the GM z heterozygotes was higher (51.1 vs 26.7%; $p = 0.006$; OR $= 2.8$) in SSc patients with IgG antibodies to HCMV than in subjects who lacked these antibodies. These genotypes were not associated with the prevalence of anti-HCMV antibodies in the control population. Also, KM and GM n genotypes were not significantly associated with the prevalence of anti-HCMV antibodies (data not shown).

The prevalence of IgG HCMV antibodies (Table II) as well the distribution of GM f/z genotypes (Table III) in SSc patients—as a whole or when subdivided into diffuse and limited form of the disease—was not significantly different from that in controls.

**Discussion**

The results presented here show that neither the prevalence of IgG HCMV antibodies nor the presence of particular GM genotypes, by itself, is a risk factor for the development of scleroderma. However, in patients with the disease, the occurrence of anti-HCMV antibodies is strongly associated with particular GM genotypes. SSc patients homozygous for the GM f allele were 60% less likely to be seropositive than those homozygous for the z allele or the heterozygotes. Conversely, those heterozygous for the GM f and z alleles were almost three times more likely to be seropositive than the subjects with the other two genotypes.

The lack of association between GM markers and SSc observed here is consistent with the results of our earlier studies (3, 4). These markers, like the HLA determinants, have been shown to contribute more significantly to the generation of particular autoimmune responses in SSc rather than to the onset of the disease itself (3, 4, 9). The significant association between the heterozygosity at the GM locus and antibody responsiveness to HCMV observed here may be explained by molecular mimicry. In SSc patients heterozygous for the f and z alleles, infection with HCMV may generate a host antiviral response that is cross-reactive with certain autoantigens. Perhaps the B cells carrying the f/z phenotype on their Ig receptors in SSc patients are more efficient in the uptake, processing, and subsequent presentation of certain SSc peptides (with significant structural homology to HCMV epitopes) to the collaborating T cells, resulting in autoantibody production. It is relevant to note that Lunardi et al. (2), after screening a random peptide library with pooled IgG antibodies from SSc patients, iden-

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**Table I. Distribution of GM f,z genotypes in scleroderma patients and controls.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients (n = 135)</th>
<th>Controls (n = 141)</th>
</tr>
</thead>
<tbody>
<tr>
<td>f</td>
<td>38 (42.2%)</td>
<td>28 (62.2%)</td>
</tr>
<tr>
<td>z</td>
<td>6 (6.7%)</td>
<td>5 (11.1%)</td>
</tr>
<tr>
<td>f,z</td>
<td>46 (51.1%)</td>
<td>12 (26.7%)</td>
</tr>
</tbody>
</table>

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**Table II. Prevalence of IgG antibodies to HCMV in scleroderma patients and controls.**

<table>
<thead>
<tr>
<th></th>
<th>Patients (N = 137)</th>
<th>Controls (N = 145)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCMV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seropositive</td>
<td>91 (66.4%)</td>
<td>100 (69.0%)</td>
</tr>
<tr>
<td>Seronegative</td>
<td>46 (33.6%)</td>
<td>45 (31.0%)</td>
</tr>
</tbody>
</table>

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**Table III. Distribution of GM f,z genotypes in scleroderma patients and controls.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients (n = 135)</th>
<th>Controls (n = 141)</th>
</tr>
</thead>
<tbody>
<tr>
<td>f</td>
<td>66 (48.9%)</td>
<td>63 (44.7%)</td>
</tr>
<tr>
<td>z</td>
<td>11 (8.1%)</td>
<td>17 (12.1%)</td>
</tr>
<tr>
<td>f,z</td>
<td>58 (43.0%)</td>
<td>61 (43.3%)</td>
</tr>
</tbody>
</table>
tified a peptide that had considerable sequence homology with several autoantigens (e.g., cytochrome C and fibril- 
larin) as well as to the HCMV protein UL94. Affinity purified antibodies to this peptide isolated from the sera of 
SSc patients, but not those isolated from the normal subjects, cross-reacted with the autoantigens and the HCMV 
protein UL94. Thus, it appears that the antibodies produced against HCMV in SSc patients may be qualitatively dif-
ferent — possibly recognizing different epitopes of HCMV from those recognized by the control subjects — and 
subject to GM allotype restriction. Although GM markers are constant-
region determinants, there is a growing body of evidence for the involvement of these regions in the antibody specific-
ity usually associated with the variable region of the Ig molecule. Possible mechanisms include a direct contribution 
to the formation of idiotypic determinants, modulation of antibody bind-
ing affinity, and linkage disequilibrium with alleles coding for the variable-
region epitopes (10-12). It is especially noteworthy that the CH1 domain of the 
\( \gamma_1 \) — where the allelic determinants GM \( f \) and \( z \) are located — has been shown to modulate the kinetic compe-
tence of antigen binding sites (13). GM allotypes may also influence autoanti-
body responses to the viral epitopes through differential binding of the IgG molecule to the Fc receptor (FcR). Evi-
dence for such allotypic restriction in the binding affinity of the IgG1 mole-
cules to the FcR has been presented for the herpes simplex virus type 1 (14). HCMV too encodes for an IgG Fc-
binding protein (15) and the binding af-
finity of this protein may be similarly influenced by the GM determinants present on the IgG molecule. 
The lack of a significant difference in the prevalence of IgG HCMV antibo-
dies between patients and controls ob-
served in this study is in variance with the findings of Neidhart et al. (16), who reported an elevated prevalence of these 
antibodies in SSc patients. The reasons for this discordance are not clear; pos-
sible explanations include the differences in the ethnic background of the study populations and the choice of con-
trols in the two studies. The genetic background of the Swiss subjects studied by Neidhart et al. is probably more 

equatorial compared to the south-
eastern U.S. population in the present investigation. Also, a significant propor-
tion of controls in the study by Neid-
hart et al. were patients with rheuma-
toid arthritis; in the present investiga-
tion, all controls with autoimmune or 
connective tissue diseases were exclud-
ed. In view of the reported cross-reactivity 
between anti-HCMV antibodies and various autoantigens in SSc patients (2), it would be interesting to determine whether autoantibody responses to these antigens — especially to the au-
toantigen NAG-2, which is highly ex-
pressed on endothelial cells — are in-
fluenced by GM genes. This is the first 
report, to my knowledge, implicating GM 
genes in antibody responses to HCMV. 

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