Autoantibodies to RuvBL1 and RuvBL2: a Novel Systemic Sclerosis-Related Antibody Associated with Diffuse Cutaneous and Skeletal Muscle Involvement

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Introduction

Systemic sclerosis (SSc)

Serum anti-nuclear antibodies (ANAs) are detected in more than 95% of SSc patients. A variety of ANAs have been reported to be specific for SSc. These antibodies are associated with distinct clinical subsets, and are present at diagnosis and almost mutually exclusive to each other. These SSc-related autoantibodies are identified in 80% of entire SSc population, but in other words, there are still 20% of patients without these known antibodies.

Patients (Kanazawa Univ) (1995 - 2009)

We first examined specificity of anti-ps2/48 antibody in sera from patients with various connective tissue disease, interstitial lung disease alone without definitive diagnosis of connective tissue disease, and autoimmune hepatitis in the Kanazawa cohort as well as healthy control sera. Anti-ps2/48 antibody was detected in 6 (1.9%) of 316 SSc patients, but not in any of patients with other diseases or healthy controls. This indicates that anti-ps2/48 antibody is highly specific to SSc.

Disclosure

I have no relevant financial relationships to disclose.
The prevalence of anti-p52/48 ab in the two cohorts was 1.5% and 1.1%, respectively, in consecutive SSc patients. Anti-p52/48 antibody is a rare SSc-specific antibody, whose prevalence is 1-2% in three independent cohorts.

Indirect immunofluorescent staining

An indirect immunofluorescent staining pattern on Hep-2 cell slide by a SSc serum positive for anti-p52/48 ab. All sera positive for anti p52/48 ab commonly produced a speckled nuclear pattern at a titer ranging from 1:160 to 1:1280. This pattern was characterized by condensation of staining during prophase, followed by attenuation of staining in the chromosomal area of metaphase mitotic cells.

RuvBL1, RuvBL2

RuvBL1 and RuvBL2 are ATP-binding proteins that belong to the AAA+ (ATPase associated with diverse cellular activities) family. RuvBL1 is present in the nucleus as complex with RuvBL2. These proteins express ubiquitously. The molecular weights of RuvBL1 and RuvBL2 are reported to be 49 and 48 kDa.

Detection of RuvBL1/2 in immunoprecipitates using immunoblots

Protein components immunoprecipitated from the cellular extracts by 3 representative anti-p52/48-positive SSc sera were subjected to immunoblots probed with anti-RuvBL1 and anti-RuvBL2 monoclonal antibodies. As you can see in the slide, anti-RuvBL1 and anti-RuvBL2 antibodies reacted with the immunoprecipitates by anti-p52/48 positive sera. Therefore the 50kDa doublet was confirmed to be RuvBL1 and RuvBL2.
We have identified a novel anti-RuvBL1/2 autoantibody in two Japanese cohort combined and Pittsburgh cohort.

**Anti-RuvBL1/2**

<table>
<thead>
<tr>
<th>Organ involvement</th>
<th>Negative (n = 374)</th>
<th>Positive (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative (n = 458)</td>
<td>Positive (n = 27)</td>
<td></td>
</tr>
<tr>
<td>Pittsburgh cohort</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scleroderma</td>
<td>534 (90)</td>
<td>10 (100)</td>
<td>0.8</td>
</tr>
<tr>
<td>Kidney (renal crisis)</td>
<td>207 (40)</td>
<td>215 (47)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Heart</td>
<td>17 (3)</td>
<td>57 (10)</td>
<td>0.03/0.02</td>
</tr>
<tr>
<td>Kidney</td>
<td>57 (10)</td>
<td>58 (100)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

**Values are the number (percentages) unless indicated otherwise.**

In summary:

- We have identified a novel SSc-related autoantibody reactive with a complex consisting of RuvBL1 and RuvBL2.
- Anti-RuvBL1/2 was exclusively detected in SSc patients, but its prevalence is very low, ranging from 1% to 2%.
- SSc patients with anti-RuvBL1/2 in both Japanese and Pittsburgh cohort had higher frequencies of SSc in overlap with myositis and dcSSc than those without.
- Compared with other autoantibodies related to SSc-myositis overlap, anti-RuvBL1/2 was distinctive in terms of its associations with older age at SSC onset and male sex.
- Detection of anti-RuvBL1/2 antibody was useful for diagnosis and myositis overlap in SSc.