Interstitial lung disease in systemic sclerosis is associated with autoimmunity to α1(V) chain of type V collagen

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Systemic sclerosis (SSc) is a chronic autoimmune disease, characterized by vascular damage, inflammation and fibrosis of the skin and internal organs.

Interstitial lung diseases (ILD) are among the most serious complications associated with SSc.

The identification of biomarkers could predict the course of the disease.
Type V collagen (Col V)

- Col V is a fibrillary protein
- Col V isoform [α1(V)2, α2(V)] is located embedded within collagen I, III and V heterotypic fibrils
- Col V can be considered a sequestered antigen
- Potential to become an autoantigen when exposed to the immune system

- ColV has been associated to autoimmunity in pulmonary fibrosis
- Our group have shown the pathogenic role of ColV in clinical and pre-clinical models of SSc.
Objective

Our aim was to evaluate the autoimmunity to α1(V) and α2(V) chains of the Col V in SSc-ILD
**Sera samples**
- Patients with Early-SSc (n=19)
  - Patients-EULAR Preliminary Criteria
- Health individuals (n=6)

**Lung biopsies**
- SSc-ILD patients (n=4)
  - Histologic pattern of NSIP
- Control individuals (n=6)

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**Evaluation of the α1(V) and α2(V) chains immunogenicity in SSc**

- Early SSc patient sera samples positive to anti-Col V (n=7)
  - Isolate IgG
  - Early-SSc anti-ColV-IgG+
    - Biotinylated
  - Immunofluorescence

- Early-SSc Biotinylated Anti-ColV-IgG+
  - Adsorption
  - α1(V) chain
    - Biotinylated Early-SSc anti-ColV-IgG+/ads-α1(V)
  - Immunofluorescence
  - α2(V) chain
    - Biotinylated Early-SSc anti-ColV-IgG+/ads-α2(V)

- Pulmonary tissue of SSc-ILD
  - Biotinylated ColV-IgG+
  - Fluorescent Streptavidin
## Evaluation of immunogenic peptides of the Collagen V in Early-SSc sera samples

### Immunogenic Col V peptides

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Amino acids sequence</th>
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<tbody>
<tr>
<td>Col5A1(599)</td>
<td>PPGPAGKPGRGG</td>
</tr>
<tr>
<td>Col5A1(779)</td>
<td>GIRGLKGTKGKGED</td>
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<tr>
<td>Col5A1(909)</td>
<td>RGQRGPTGPRGERGPRG</td>
</tr>
<tr>
<td>Col5A1(1.049)</td>
<td>KDGPPGLRGFPDGRG</td>
</tr>
<tr>
<td>Col5A1(1.439)</td>
<td>LRGIPGPVGEQGLPG</td>
</tr>
<tr>
<td>Col5A2(275)</td>
<td>PGEVFAGSPFARGF</td>
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<tr>
<td>Col5A2(419)</td>
<td>PGAIGTDGTPGAKGP</td>
</tr>
<tr>
<td>Col5A2(1.078)</td>
<td>NIRFRYIVLQDTCSK</td>
</tr>
</tbody>
</table>


### Flow cytometry assay

- Early-SSc sera samples with anti-ColV antibodies (n=7)

- Reaction was revealed with a fluorescent conjugate and analyzed in Flow Cytometer
Lung Histologic Characterization

Control pulmonary tissue

SSc-ILD patients pulmonary tissue

NSIP histological pattern
Immunofluorescence with Early-SSc anti-ColV-IgG+ in pulmonary tissue

Control pulmonary tissue

- Weak green fluorescence in the vascular wall

SSc-ILD pulmonary tissue

- ↑ immunostaining in vascular layers
- ↑ immunostaining along the thickened alveolar septa
- ↑ immunostaining along of the basal and adventitial layers in the bronchioles
Evaluation of co-location of the Early-SSc anti-ColV-IgG+ and Col V in pulmonary tissue

Control pulmonary tissue  
SSc-ILD pulmonary tissue

Rabbit Anti-human Col V antibody

Col V / Red

Early-SSc anti-ColV_IgG+ / green

Merge

Co-location of the Col V and anti-ColV-IgG+

-Vessels and bronchioles basal layers and along the alveolar septa in SSc-ILD
Results

After adsorption of Early-SSc anti-ColV-IgG+ with α(V) chains

Control pulmonary tissue

SSc lung: ColV-IgG+

CT lung: ColV-IgG+

1(V)α

SSc lung: ColV-IgG+/ads−

1(V)α

CT lung: ColV-IgG+/ads−

2(V)α

SSc lung: ColV-IgG+/ads−

2(V)α

CT lung: ColV-IgG+/ads−

0

10

20

30

40

50

Immunofluorescence intensity (%)

ColV-IgG+/ads-α2(V) – immunostaining in vessel layers and along the thickened alveolar septa
**Flow cytometry**

Positive reactivity to immunogenic Col V peptide in control and Early-SSc patient sera
Antibodies detection to Col V peptides in early-SSc patients sera

Results

Flow cytometry

-Early-SSc Anti-Col V sera samples - immunoreactivity for Col5A1(599) and Col5A1(1,049) peptides
Conclusion

- We found that the autoimmunity to Col V in Early-SSc is directed to α1(V) chain, emerging as promisor biomarker of prognosis and target therapy in SSc-ILD patients

- The two immunogenic α1(V) chain peptides [Col5A1(599) and Col5A1(1.439)] probably are related to autoimmunity to α1(V) chain in SSc
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