Chemokine-like Factor Expression in the Idiopathic Inflammatory Myopathies

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Introduction

Idiopathic inflammatory myopathies are the most common forms of acquired myopathies that includes:

- Polymyositis (PM),
- Dermatomyositis (DM)
- Inclusion body myositis
The suggested diagnostic features include

- Symmetrical proximal muscle weakness
- Elevation of serum skeletal muscle enzymes
- Specific electromyographic triad
- Characteristic muscle biopsy abnormalities
- Typical skin rash of DM
In the pathogenesis of inflammatory myopathies, the following signaling molecules governing leukocyte activation and migration have been elucidated:

- Intercellular adhesion molecule
- Vascular cell adhesion molecule
- Veukocyte function-associated antigen
- Very late activated antigen
- Tumor necrosis factor-α (TNF-α)
- Interferon-γ (IFN-γ)
- Transforming growth factor-β (TGF-β)
Recently, a novel cytokine, chemokine-like factor (CKLF) containing CC motif has been identified and characterized.

CKLFs, has a role in regulation of human skeletal muscle during myogenesis

Thus, in present study we evaluated the expression of chemokine-like factor (CKLF) in biopsied muscle fibers.

- Inflammatory myopathies
- Non-inflammatory myopathies
- Neurologically diseased patients
Materials and methods

- Clinical materials
  - Four groups of patients were studied
    - The first group (n=15) with polymyositis,
    - second group (n=5) with dermatomyositis
    - Third group (n=15) muscular dystrophies
    - Fourth group (n=9) neurological diseased patients

- Immunohistochemistry of biopsied muscles
- Muscle cell culture
- Reverse Transcription Polymerase Chain Reaction (RT-PCR)
- Western blot analysis
Results

- Immunohistochemistry of biopsied muscle fibers

<table>
<thead>
<tr>
<th></th>
<th>Polymyositis</th>
<th>Dermatomyositis</th>
<th>Muscular dystrophies</th>
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<tbody>
<tr>
<td>CKLF</td>
<td>![Image]</td>
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<tr>
<td>MHC-d</td>
<td>![Image]</td>
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<tr>
<td>LFA-1</td>
<td>![Image]</td>
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CKLF immunoreactivities were remarkably detected in the inflammatory myopathies patients (14 PM and 4 DM), but not in the biopsies of non-inflammatory myopathies (n=15) and neurologically diseased patients (n=9).

CKLF was mainly detected in small diameter (usually less than 5 μm) muscle fibers.

CKLF-positive muscle fibers was more than that of MHC-d-positive muscle fibers.
Expression of CKLF in muscle cell culture

- Initially the myoblasts in muscle cell culture showed positive immunoreactivity for CKLF.
- Immunoreactivities were abolished differentiated multinucleated myotubes.
- Treatment with thrombin obtained immunoreactivity for CKLF.
Expression CKLFs and PARs in myotubes by RT-PCR

Thrombin treatment increased CKLF-1, CKLF-4 and CKLF-2 and PAR-1 expressions dose-dependently.
Up-regulation of CKLF gene expression mediated by PKC activation

<table>
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<tr>
<th>Myoblast</th>
<th>Myotube</th>
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<tbody>
<tr>
<td>1 2 3 4 5</td>
<td>6 7 8 9 10</td>
</tr>
<tr>
<td>CKLF 2</td>
<td>CKLF 2</td>
</tr>
<tr>
<td>CKLF 4</td>
<td>CKLF 4</td>
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<tr>
<td>CKLF 1</td>
<td>CKLF 1</td>
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<td>GAPDH</td>
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<tr>
<th>Thrombin</th>
<th>Staurosporin</th>
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<tr>
<td>(-) (+) (+)</td>
<td>(-) (+) (+)</td>
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<td>(-) (-) (+)</td>
<td>(-) (-) (+)</td>
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- In myoblasts, mRNA of CKLF was expressed constitutively.
- In myotubes, mRNA of CKLF is inducible and PKC-dependent.
Effect of protein kinase inhibitor on thrombin-induced CKLF up-regulation by Western blot analysis

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<tr>
<th></th>
<th>Myoblast</th>
<th>Myotube</th>
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<tr>
<td>18 kDa</td>
<td><img src="386x337.png" alt="Image" /></td>
<td><img src="552x377.png" alt="Image" /></td>
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<tr>
<td>Thrombin</td>
<td>(-) (+) (+)</td>
<td>(-) (+) (+)</td>
</tr>
<tr>
<td>Staurosporin</td>
<td>(-) (-) (+)</td>
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In myoblasts and myotubes, control and thrombin treatment showed a clear band below 18 kDa, staurosporine treatment reduced that expression.
Discussion

- CKLF was remarkably expressed in the muscles from DM and PM as compared with non-inflammatory myopathies and neurologically diseased patients.

- CKLF might be used as a marker for muscle regeneration in inflammatory myopathies.

- There may be a role of CKLF to chemoattract lymphocytes at the site of inflammation and regenerating muscle fibers.
Thrombin might play a major role for CKLF expression in the regenerating muscle fibers in inflammatory myopathies.

CKLF was expressed constitutively in myoblasts, but was inducible by thrombin in myotubes.

Our study provides a new insight of thrombin involvement in the immunopathogenesis of inflammatory myopathies and the expressed CKLF can be used as a novel marker for regenerating muscle fibers during muscle inflammation.
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