Autoantibodies in Idiopathic Inflammatory Myopathies

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Idiopathic Inflammatory Myopathies (IIM)

• Heterogeneous group of systemic *autoimmune* syndromes characterized by *chronic muscle weakness* and striated *muscle inflammation*

• Polymyositis (PM)
• Dermatomyositis (DM)
• Inclusion body myositis (IBM)
• Necrotising Autoimmune Myositis (NAM)
Clinical features

• Systemic conditions with predominant manifestations on skeletal muscle
  
  • Muscle
  • Joints
  • Lungs – interstitial lung disease
  • GIT
  • Cardiac

• Patterns of muscle weakness
  
  • PM/DM: symmetrical proximal upper and lower limbs, neck flexors
  • IBM: (asymmetrical) quadriceps weakness ≥ hip flexors, long finger flexors
  • dysphagia
  • DM: cutaneous features
Diagnosis of IIM

- Clinical presentation
- Raised serum muscle enzymes – CK
- Electromyography: myopathic triad
- Muscle biopsy
  - definitive diagnostic test
  - Used to categorise disease

*Increasing interest in the role of autoantibodies in classification and prognostication*
Role of antibodies in IIM

• Directed to nuclear and cytoplasmic antigens involved in protein synthesis
• Several strong associations between autoantibodies and clinical phenotypes
• Diagnostic markers for disease
• Divide patients into homogeneous subgroups

• Proposals for serological classification of IIM
Negative ANA Does Not Imply Antibody Negativity

Dimitri, Muscle and Nerve, 2007

Homogeneous, diffuse cytoplasmic staining
## Myositis-Associated Autoantibodies

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Antigen</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM-Scl</td>
<td>Unidentified</td>
<td>PM/ DM/ SSc overlap syndrome</td>
</tr>
<tr>
<td>U1-RNP</td>
<td>U1 small RNP</td>
<td>MCTD</td>
</tr>
<tr>
<td>Ro52</td>
<td>RNA protein TRIM21</td>
<td>IIM, pSS, SLE &amp; ILD</td>
</tr>
<tr>
<td>Ku</td>
<td>DNA-binding proteins</td>
<td>DM/PM with SLE/SSc overlap</td>
</tr>
</tbody>
</table>
# Myositis-Specific Autoantibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Target</th>
<th>Subset</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetases</td>
<td>ARS</td>
<td>PM/DM</td>
<td>Anti-synthetase syndrome</td>
</tr>
<tr>
<td>Mi-2</td>
<td>NuRD</td>
<td>DM</td>
<td>Shawl, V-neck, Gottron’s</td>
</tr>
<tr>
<td>SRP</td>
<td>SRP 72, 54 kDa</td>
<td>PM/NM</td>
<td>Severe/refractory NM</td>
</tr>
<tr>
<td>SAE</td>
<td>SUMO</td>
<td>DM</td>
<td>ILD, dysphagia</td>
</tr>
<tr>
<td>NXP2</td>
<td>NXP-2</td>
<td>JDM</td>
<td>Calcinosis, ulceration</td>
</tr>
<tr>
<td>TIF-1γ</td>
<td>TIF1γ (p155/140)</td>
<td>DM, JDM</td>
<td>Severe skin, malignancy</td>
</tr>
<tr>
<td>MDA-5</td>
<td>MDA-5</td>
<td>DM</td>
<td>Amyopathic, ILD</td>
</tr>
<tr>
<td>HMGCR</td>
<td>HMGCR</td>
<td>IMNM/NAM</td>
<td>Necrotizing myopathy</td>
</tr>
</tbody>
</table>
## Anti-synthetase antibodies

<table>
<thead>
<tr>
<th>Anti-synthetase Ab</th>
<th>tRNA synthetase</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Jo-1</td>
<td>Histidyl</td>
<td>PM, DM +ILD</td>
</tr>
<tr>
<td>Anti-PL-7</td>
<td>Threonyl</td>
<td>PM, DM +ILD</td>
</tr>
<tr>
<td>Anti-PL-12</td>
<td>Alanyl</td>
<td>ILD&gt; myositis</td>
</tr>
<tr>
<td>Anti-EJ</td>
<td>Glycyl</td>
<td>PM&gt;DM +ILD</td>
</tr>
<tr>
<td>Anti-OJ</td>
<td>Isoleucyl</td>
<td>ILD +PM/DM</td>
</tr>
<tr>
<td>Anti-KS</td>
<td>Asparaginyl</td>
<td>ILD&gt; myositis</td>
</tr>
<tr>
<td>Anti-Zo</td>
<td>Phenylalanyl</td>
<td>ILD +PM/DM</td>
</tr>
<tr>
<td>Anti-Ha</td>
<td>tyrosyl</td>
<td>ILD +PM/DM</td>
</tr>
</tbody>
</table>
Anti-Jo-1 Autoantibody

- Directed against histidyl-tRNA synthetase
- Ag: enzyme that catalyzes binding of an amino acid to its tRNA in process of protein synthesis
Anti-synthetase syndrome

- PM or DM
- Interstitial Lung Disease
- Fever
- Arthritis
- Raynauds phenomenon
- Mechanic’s hands
Jo-1 versus non-Jo-1 antisynthetases

• Jo-1
  • more likely muscle involvement
  • arthritis

• Non-Jo1
  • more likely ILD, CTD overlap
  • Raynaud’s phenomenon more common

• Differences between each of the non Jo-1 antisynthetases
  • OJ – arthritis prominent, ILD then myositis
  • EJ: Heliotrope, Gottrons
  • KS: increase CK
South Australian Myositis Database – Autoantibodies detected in 32%

- Biopsy-proven cases of IIM subsequent to 1980
- Central reporting of all adult muscle biopsies in SA in Neuropathology Lab
- DM, PM, IBM, necrotising myopathy
- DNA and serum stored

- Autoantibodies present in 42/130 (32%) myositis patients
Antibodies to Ro52 were the commonest
Ab are more common in DM than PM or IBM

Frequency of Ab mirrored the frequency of DR4

DM (11/17) cf PM (23/70), p = 0.033

DM cf IBM (8/43 =p = 0.002)
### Myositis Ab are associated with both HLA DR3 and DR4

Previous reports - linked DR3 with MSA formation in IIM patients.
(Arnett FC, Arthritis Rheum 1996;39(9):1507-18)

<table>
<thead>
<tr>
<th>DRB1</th>
<th>Autoantibody</th>
<th>Odds Ratio</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos (n=37)</td>
<td>Neg (n=81)</td>
<td></td>
</tr>
<tr>
<td>DRB1*03</td>
<td>23 (31%)</td>
<td>23 (14%)</td>
<td>3.3 (1.7, 6.6)</td>
</tr>
<tr>
<td>DRB1*04</td>
<td>13 (18%)</td>
<td>12 (7%)</td>
<td>3.6 (1.5, 8.6)</td>
</tr>
<tr>
<td>Other</td>
<td>38</td>
<td>127</td>
<td>1</td>
</tr>
</tbody>
</table>

DR3 and DR4 are both systematically assoc with autoantibody production in IIM

Limaye V et al, Rheumatol Int. 2012;32(3):611-9
Antibodies in DM
Anti-Mi2

• 11-59% prevalence in DM
• Skin manifestations
• relatively mild disease
• less internal organ involvement
• treatment response - fair
• latitudinal gradient (UV intensity)
Novel Autoantibodies in DM

• Ab in DM often assoc with distinct clinical phenotypes
• Tend to be mutually exclusive
  • specific immune responses may shape different phenotypes

• MDA5
• TIF1 Γ
• NXP2
• SAE
Antibodies to MDA5

• Target antigen: melanoma differentiation-associated gene 5
• 10-48% Asians, 0-13% Caucasians
• Clinically
  • Rapidly progressive ILD
    • Sato, Arthritis Rheum 2005
  • Novel cutaneous phenotype
    • palmar papules
    • cutaneous ulcerations
    • severe vasculopathy
  • Amyopathic DM
• HLA–DRB1*08
Clinical phenotype of IIM with Anti-MDA5

Fiorentino, J Am Acad Derm, 2011
Antibodies to TIF1γ

- Target antigen: transcriptional intermediary factor 1-γ
- Originally reported as anti-p155/p140
- 13-31% DM
- Adults: Ca-associated DM
  - Sensitivity for Ca 78%
  - Specificity for Ca 80%
- Less Raynauds, calcinosis and ILD
- Juvenile DM: no malig but skin ulceration
- DQA1*0301 association
Antibodies to TIF1γ: manifestations according to age

- **Children**
  - Ulceration/vasculitis

- **Young adults**
  - Rash
  - Amyopathic

- **Older adults**
  - Malignancy
  - Myositis
# Frequency of Anti-MDA5 and Anti-TIF1γ

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Mean (SD)</th>
<th>M:F</th>
<th>Total</th>
<th>Anti-MDA5 (%)</th>
<th>Anti-TIF1γ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>3-84</td>
<td>50 (19)</td>
<td>24:58</td>
<td>82</td>
<td>21 (26)*</td>
<td>12 (15)</td>
</tr>
<tr>
<td>CADM</td>
<td>3-84</td>
<td>48 (20)</td>
<td>7:24</td>
<td>31</td>
<td>20 (65)**</td>
<td>3 (10)</td>
</tr>
<tr>
<td>CA-assoc DM</td>
<td>48-80</td>
<td>66 (11)</td>
<td>5:7</td>
<td>12</td>
<td>0</td>
<td>7 (58)***</td>
</tr>
<tr>
<td>Classical DM</td>
<td>16-76</td>
<td>47 (17)</td>
<td>12:27</td>
<td>39</td>
<td>1 (3)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>PM</td>
<td>32-70</td>
<td>57 (14)</td>
<td>0:6</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SLE</td>
<td>15-76</td>
<td>50 (15)</td>
<td>5:16</td>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SSc-ILD</td>
<td>30-75</td>
<td>58 (10)</td>
<td>3:23</td>
<td>26</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Controls</td>
<td>46-72</td>
<td>54 (6)</td>
<td>4:16</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cancer</td>
<td>48-78</td>
<td>58 (7)</td>
<td>5:15</td>
<td>21</td>
<td>NA</td>
<td>0</td>
</tr>
</tbody>
</table>

*P<0.05 in DM vs SLE, SSc-ILD, healthy controls. **P<0.005 in CADM vs ca-assoc DM or classical DM without cancer by a chi-square test. ***P<0.005 in cancer-associated DM vs CADM or classical DM without cancer

Hoshino K et al, Rheumatology, 2010
Antibodies to NXP2

• Ag: 140kDa nuclear matrix protein-2
• Frequency
  • <5% adult DM
  • JDM 23-25%
• Most frequent Ab in Italian cohort (17%)
• In JDM:
  • ↑ risk of calcinosis
  • ↑ disease severity

Gunawardena H. Arthritis Rheum 2009
Ceribelli A. Arthritis Res Ther 2012
Antibodies to SAE

- **Ag**: small ubiquitin like modifier activating enzyme (SAE)
- **Frequency**
  - <5% adult DM
  - <1% JDM
- **Clinically**
  - Often cutaneous features first
  - Mild muscle involvement
  - Dysphagia
- **Low freq malignancy and ILD**
- **HLA-DRB1*04-DQA1*03-DQB1*03**
  - Betteridge ZE. Ann Rheum Dis 2009
Low frequency of novel antibodies

Hungarian cohort IIM n=337

- 12 anti-TIF1g
- 4 anti-NXP2
- 4 anti-SAE
- 0 anti-MDA5

SA Myositis Registry n=193

- 3 TIF1g
- 1 anti-NXP2
- 0 anti-MDA5

- Together with Neil McHugh, Zoe Betteridge, Bath, UK

- Bodoki L et al Autoimmune Rev 2014
Antibodies in IBM
**Anti-cN1A**

- 43kDa muscle autoantigen: cytoplasmic 5’-nucleotidase 1A (cN1A)
- Strengthens role for B-cell mediated autoimmunity in IBM
- IgG anti-cN1A :>90% specificity and 34-70% sensitivity in IBM
- Detection of multiple isotypes increased sensitivity to 76%
  - Greenberg SA Muscle Nerve 2014
- cN1A accumulates in perinuclear regions and rimmed vacuoles in IBM muscle and localises to areas of myonuclear degeneration
- ? Provide a link between dual processes of autoimmunity and myodegeneration
  - Larmen B et al Ann Neurol 2013
- ? Biomarker for IBM
Anti-cN1A: SA Myositis Database

• Detected in 24/69 (35%) patients with IBM*
• IgM isotype most frequent (n=17), IgG (n=13) and IgA (n=5)
• No gender difference: Ab+ve 15/24 female, Ab neg : 27/45 female
• No diff in frequency of malignancy in patients with anti-cN1A (3/20) compared to those without (10/39), p=0.51
• Antibodies to other MSA/MAA were present in a minority (8/56) of patients with IBM and were significantly less prevalent than anti-CN1A (p=0.01)

Necrotising autoimmune myositis

anti-SRP
anti-HMGCR
Antibodies to signal recognition particle (SRP)

- Ribonucleoprotein – targets secretory proteins to endoplasmic reticulum
- Anti-SRP detected in 4-6% of patients with myositis

- Clinically
  - Rapidly progressive weakness
  - Marked elevation of CK
  - Cardiac involvement
  - Muscle biopsy typically shows necrotizing myopathy
  - Traditionally - poor prognosis/ response to treatment
Anti-HMGCR and necrotising autoimmune myositis

- Statins can trigger an immune-mediated necrotizing myopathy which persists despite statin discontinuation
  - suggests immune mechanisms involved

- Statins up-regulate HMGCR
- Regenerating muscle fibres express high levels of HMGCR
- linked with anti-100kDa proteins –since identified as HMGCR
Anti-HMGCR antibodies – what is already known?

• Detected in 6% of 750 patients with suspected IIM (Johns Hopkins Centre)

• Rarely detected in patients on statins with self-limited MSK symptoms

• Testing for anti-HMGCR by ELISA
  • high sensitivity (94%)
  • high specificity (99%)

• Levels of anti-HMGCR correlate with CK levels and proximal weakness

• Anti-HMGCR persists despite clinical improvement following immunosuppressive therapy.

• Testing for anti-HMGCR - proposed to be useful diagnostically in patients with suspected statin-mediated immune necrotizing myopathy
SA Myositis Database: Anti-HMGCR detected 9% IIM/ NM

• Detected in 19/207 (9.2%) sera from patients with IIM/NM*

• Anti-HMGCR was not detected in any of 151 sera from a general reference Western Australian Busselton population.
Anti-HMGCR is equally distributed among IIM subsets

<table>
<thead>
<tr>
<th>IIM Subgroup</th>
<th>Anti-HMGCR +ve</th>
<th>Anti-HMGCR -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (n=26)</td>
<td>1 (4%)</td>
<td>25 (96%)</td>
</tr>
<tr>
<td>PM (n=74)</td>
<td>8 (11%)</td>
<td>66 (89%)</td>
</tr>
<tr>
<td>IBM (n=62)</td>
<td>6 (10%)</td>
<td>56 (90%)</td>
</tr>
<tr>
<td>IIM NOS (n=13)</td>
<td>1 (8%)</td>
<td>12 (92%)</td>
</tr>
<tr>
<td>Necrotizing (n=23)</td>
<td>2 (9%)</td>
<td>21 (91%)</td>
</tr>
<tr>
<td>Other (n=9)</td>
<td>1 (11%)</td>
<td>8 (89%)</td>
</tr>
<tr>
<td>Total (n=207)</td>
<td>19 (9%)</td>
<td>188 (91%)</td>
</tr>
</tbody>
</table>

The prevalence of anti-HMGCR was comparable among subsets of IIM (p=0.95).
## Associations of anti-HMGCR

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Anti-HMGCR +ve</th>
<th>Odds Ratio (95% CI)</th>
<th>p-value</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statin use</td>
<td>16/52 (31%)</td>
<td>39 (9, 361)</td>
<td>&lt;10^-8</td>
<td>0.31 (0.19, 0.45)</td>
<td>0.99 (0.96, 1)</td>
</tr>
<tr>
<td>No statin use</td>
<td>1/130 (0.1%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR11 positive</td>
<td>10/24 (42%)</td>
<td>50 (11, 486)</td>
<td>&lt;10^-8</td>
<td>0.42 (0.22, 0.63)</td>
<td>0.99 (0.95, 1)</td>
</tr>
<tr>
<td>DR11 negative</td>
<td>1/105 (0.1%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR11+ve &amp; statin</td>
<td>9/10 (90%)</td>
<td>80 (10, 1108)</td>
<td>&lt;10^-7</td>
<td>0.90 (0.55,1)</td>
<td>0.95 (0.75, 1)</td>
</tr>
<tr>
<td>DR11 –ve &amp; statin</td>
<td>1/20 (5%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>11/77 (14%)</td>
<td>2.5 (1.0, 6.6)</td>
<td>0.079</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>8/130 (6%)</td>
<td></td>
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</tbody>
</table>

Limaye V et al. Muscle Nerve 2014
Anti-HMGCR antibodies – role of statins?

• Among anti-HMGCR positive pts, preceding statin exposure in
  • 24/26 (92.3%)  Mammen A et al 2011
  • 20/45 (44.4%)  Allenbach et al Medicine 2014
  • 16/19 (84%)  Limaye et al 2014

• Statins $\uparrow$ HMGCR expression  DRB1*1101  Anti-HMGCR immune response

Statin-naïve patients – what triggers disease?
  natural supplements which reduce cholesterol? Trigger HMGCR expression
Statins found in food products and supplements
  oyster mushrooms – lovastatin
  red yeast – peking duck glaze
  Other environmental triggers
Conclusions

• Detection of autoantibodies in IIM: role for B-cell mediated autoimmunity
• A number of these antibodies are under genetic control

• Disease monitoring – Do levels of autoantibodies correlate with disease activity?
• Predictive value for development of disease

• Precise role in pathogenesis
Conclusions

• Antibodies are markers for distinct clinical phenotypes
• In clinical practice autoantibodies may help to establish a diagnosis
• May prompt
  • more intensive therapy
  • Screening for associated features eg ILD, malignancy
• May enable prognostication
  • autoantibodies may correlate with disease outcome
  • Differential risk for ILD, malignancy, cutaneous features
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**Anti-cN1A**

**NXP2, TIF1g, SAE, MDA5**

**Myositis patients SA**

**Bath, UK**
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