What are Autoantibodies and how do they work in Myositis?

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Royal National Hospital for Rheumatic Diseases
Founded in 1738
Historical treatments for Arthritis
What are Antibodies?

What are Autoantibodies?
Antibodies are part of the ‘immune system’
Types of cells in the blood
Blood cells that make up the immune system

A.) Macrophage
- Engulfment of Pathogen
- Destruction

B.) B cell
- Antigen Binding
- Antibody Secretion

C.) CD8 T cell
- Infection of Skin Cell
- Virus
- Cell Killing

D.) CD4 T cell
- Antigen Presenting Cell
- Secretion of Cytokines
B cells make antibodies (immunoglobulin)
Antibodies provide protection from infection

1. The B cell finds an antigen which matches its receptors.
2. It waits until it is activated by a helper T cell.
3. Then the B cell divides to produce plasma and memory cells.
4. Plasma cells produce antibodies that attach to the current type of invader.
5. “Eater cells,” prefer intruders marked with antibodies, and “eat” loads of them.
6. If the same intruder invades again, memory cells help the immune system to activate much faster.
What are Antibodies?

What are Autoantibodies?
Antibodies and Autoantibodies

Immunoglobulin

Biological therapies

Autoantibody (anti-DNA)

LE cell first diagnostic test for SLE
What are autoantibodies?

• Antibodies (immunoglobulin produced by B cells) which instead of attaching to foreign antigens (e.g. bacteria) are directed against the host self-constituents (autoantigens)
• Most autoantibodies are not thought to be the immediate cause of disease but are ‘biomarkers’ of pathology
• Close association between particular autoantibodies and certain diseases and clinical phenotypes
• Can discriminate subgroups of patients that differ in prognosis or response to therapy.
• Autoantibody levels may reflect disease activity
Methods for detecting autoantibodies

Autoantibody Screening by Indirect Immunofluorescence

Hep-2  Human neutrophil  Hep-2  Hep-2

Autoantibody identification by second technique

Immunodiffusion  ELISA  Immunoblot  Immunoprecipitation

ENA anti-RNP  anti-PR3  anti-centromere  Anti-fibrillarin U3RNP
Indirect Immunofluorescence

• **Antigen source** - tissue section (mouse LKS, monkey oesophagus) whole cell (HEp-2, neutrophil, *crithidia luciliae*)

• **Autoantibody from patient serum** - Apply autoantibody that if present will bind to the antigen source

• **Secondary antibody** - anti-human IgG FITC

• **Visualization** - green fluorescence in a recognizable pattern corresponding to location of antigen read under a specialized immunofluorescence microscope
Indirect immunofluorescence test
Indirect immunofluorescence test II

Serum from scleroderma patient with anti-centromere autoantibodies
Indirect Immunofluorescence test III

Secondary antibody
Anti-human IgG conjugated to FITC
Indirect immunofluorescence

- If test positive the patient will be reported as having an antinuclear antibody (ANA)
- Sometimes the pattern will reveal the type of ANA (specificity) but usually another method will be necessary for exact identity
Enzyme-linked immunosorbent assay (ELISA)

1. Antigen coated well
2. Add patient serum
3. Specific autoantibody binds
4. Add enzyme-linked anti-human secondary antibody
5. Secondary antibody binds
6. Add colourless substrate
7. Substrate reacts with enzyme
8. Colour development proportional to amount of enzyme present
Immunoblot and Lineblot
Autoantibody detection by protein immunoprecipitation

Take the patient’s serum – add “beads” which binds the autoantibodies

Add the patient’s serum to the “cellular soup”

Add denaturing agent and separate proteins according to size using electrophoresis

K562 cells
Tag with radioactive material
Break up the cells to make a cellular soup

The “fingerprint” pattern will show any known and unknown autoantigens recognised by autoantibodies in the patient’s serum

Stain proteins
Autoradiography
Anti-signal recognition particle (SRP) by different assays
So what does all this mean?
The spectrum of autoimmune connective tissue disease

- Scleroderma
  - Nucleolar RNP

- Dermatomyositis
  - Transcription tators RNA synthetase

- Systemic Lupus Erythematosus
  - snRNPS
  - Nucleosome

- Rheumatoid arthritis
  - ACPA

- Granulomatous with polyangiitis
  - ANCA

- Sjogren’s
  - Ro/La (SS-A/SS-B)
How do Autoantibodies work in Myositis?
Idiopathic inflammatory Myositis Disorders
Idiopathic inflammatory myositis

- **Polymyositis**
  - Anti-synthetase syndrome
  - Immune-mediated necrotising myopathy

- **Dermatomyositis**
  - Clinically amyopathic dermatomyositis (CADM)
  - Cancer associated myositis (CAM)

- **Inclusion Body Myositis**

- **Juvenile Dermatomyositis**

- **Myositis associated with connective tissue disease**

- **Other**
  - Granulomatous, eosinophilic, focal, orbital, macrophagic, myofasciitis
Autoantibodies in myositis

• MSA (myositis ‘specific’ autoantibodies)
  • Anti-tRNA synthetases (e.g. anti-Jo-1)
  • Anti-Mi-2
  • Anti-signal recognition particle
  • Anti-SAE
  • Anti-TIF-1γ
  • Anti-MDA5
  • Anti-NXP2
  • Anti-HMGCR
  • Anti-EIF-3
  • Anti-MUP44

• MAA (myositis associated autoantibodies)
  • Anti-PM-Scl
  • Anti-U1RNP
  • Anti-Ku
  • Anti-U3RNP

MSA and MAA uncommon in malignancy-associated DM or inclusion body myositis
Patterns of juvenile versus adult myositis

- Juvenile myositis
  - JDM more common
  - Calcinosis
  - Lipodystrophy
  - Interstitial lung disease rare
  - Malignancy rare
  - Polymyositis uncommon
  - Inclusion body myositis rare
  - Overlap e.g. with scleroderma

- Adult myositis
  - Dermatomyositis
    - Association with malignancy
  - Polymyositis
    - Antisynthetase syndrome
  - Inclusion body myositis
  - Overlap

UK JDM Cohort and Biomarker Study n= 347

EUMYONET n = 1616
Autoantibodies in JDM and juvenile myositis overlap

• Until recently less well characterised
• Myositis specific autoantibodies
  • Anti-Mi-2 most frequently described
  • Low frequency of anti-synthetase and anti-SRP
• Myositis associated autoantibodies
  • Overlap syndromes with scleroderma/lupus
    • Anti-PmScl
    • Anti-U1RNP
• New MSA in JDM
  • Anti-TIF1γ
  • Anti-NXP2
  • Anti-MDA5
UK JDM Cohort and Biomarker study n = 347
Calcinitosis in juvenile dermatomyositis is influenced by both anti-NXP2 autoantibody status and age at disease onset

Sarah L. Tansley, Zoe E. Betteridge, Gavin Shaddick, Harsha Gunawardena, Katie Arnold, Lucy R. Wedderburn and Neil J. McHugh, on behalf of the Juvenile Dermatomyositis Research Group

Fig. 1 The effect of anti-NXP2 autoantibodies on the risk of calcinosis by age at disease onset (with 95% CI)

A near-linear relationship is seen between younger age at disease onset and increased risk of calcinosis.

Anti-NXP2 Autoantibodies

Children → Young Adults → Older Adults

AGE

Rash

Calcinosi

Malignancy

Anti-NXP2 Autoantibodies

Rash

Calcinosi

Malignancy
Anti-MDA5 autoantibodies in juvenile dermatomyositis identify a distinct clinical phenotype: a prospective cohort study


• Anti-MDA5 in 7.4% of JDM patients
• Associated with skin ulceration, oral ulceration and milder muscle disease
• Milder muscle biopsy in all four domains of JDM biopsy score
• 4 of 21 had interstitial lung disease

Arthritis Res Ther 2014
Summary of autoantibodies in JDM

- Myositis autoantibodies (MSA and MAA) in JDM
  - Present in 60% of cases
  - May be valuable in diagnosis
  - Newer specificities (TIF1, NXP2, MDA5) account for 40% of cases and identify clinical subsets of disease
  - Have different prevalence and associations across the myositis spectrum dependent on age of onset of disease
  - Levels may reflect disease activity
  - Provide insights into genetic and environmental mechanisms of disease
MSA/MAAs and clinical associations in adult myositis
Case A female born 1957

- **2006**
  - Breathlessness
  - 6 months later
    - Proximal muscle weakness (CK 9533 IU/L)
    - Raynaud’s
    - Arthralgia
    - Puffy fingers
  - **Non-specific interstitial pneumonia**
  - Rx Pulse methylprednisolone and IV cyclophosphamide
- **2011**
  - Mycophenolate mofetil 2.5/day and prednisolone 7.5 mg/day
Anti-synthetase syndrome: a new autoantibody to phenylalanyl transfer RNA synthetase (anti-Zo) associated with polymyositis and interstitial pneumonia

Z. Betteridge\textsuperscript{1}, H. Gunawardena\textsuperscript{1,2}, J. North\textsuperscript{1}, J. Slinn\textsuperscript{3} and N. McHugh\textsuperscript{1,2}

1. Normal Serum
2. Anti-Jo-1
3. Anti-PL-7
4. Anti-PL-12
5. Case 1 (anti-Zo)
Anti-synthetase syndrome

Clinical Features
- Myositis
- Interstitial pneumonia (50-80%)
- Arthritis (50-90%)
- Raynaud's (60%)
- Mechanics Hands (70%)
- Fever (80%)

<table>
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<tr>
<th>Autoantibody</th>
<th>tRNA synthetase target</th>
<th>Prevalence</th>
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<tr>
<td>Jo-1</td>
<td>Histidine</td>
<td>25-30%</td>
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<tr>
<td>EJ</td>
<td>Glycerine</td>
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<tr>
<td>PL-7</td>
<td>Threonyne</td>
<td>3-4%</td>
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<tr>
<td>KS</td>
<td>Asparagine</td>
<td>&lt;2%</td>
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<tr>
<td>OJ</td>
<td>Isoleucine</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>PL-12</td>
<td>Alanine</td>
<td>3-4%</td>
</tr>
<tr>
<td>Zo</td>
<td>Phenylalanine</td>
<td>&lt;2%</td>
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Learning points from Case A

• Interstitial lung disease may be the predominant or even only manifestation of myositis (anti-synthetase syndrome)
• Autoantibodies can be missed as they do not give a strong ANA on routine screening
• Multidisciplinary management is essential in myositis
• Case A would not have fulfilled older criteria (Bohan and Peter) for myositis
Case C female born 1949

• 2002
  • Rash face
  • Biopsy lupus/DM
  • Rx Predisolone and HCQ
  • 6 months later weakness and dysphagia
  • CK 797
  • ANA anti-SAE
Case C 2003-2009

Oral Prednisolone
HCQ  MTX  Cyclosp  AZA  Mycophenolate
Cyclophos/IvMP

MMDS
CPK

Infliximab

MMDS
CPK
Identification of a Novel Autoantibody in Dermatomyositis Directed Against Small Ubiquitin-like Modifier-Activating Enzyme

Zoë Betteridge,1 Harsha Gunawardena,2 Jean North,1 Jenna Slimn,3 and Neil McHugh2

<table>
<thead>
<tr>
<th>Protein Accession</th>
<th>Human Description</th>
<th>Enzyme Description</th>
<th>Score</th>
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Learning points from Case C

• Example of a case of clinically amyopathic dermatomyositis (CADM who presented with skin disease alone but later developed severe myositis

• CK was not a very useful biomarker

• Had a new autoantibody (anti-SAE) that found in about 8% of cases of DM

• Eventually made virtually full recovery
Clinical and HLA-class II haplotype associations of autoantibodies to small ubiquitin-like modifier enzyme, a dermatomyositis-specific autoantigen target, in UK adult-onset Caucasian myositis

Zoe E Betteridge, Harsha Gunawardena, Hector Chinoy, Jean North, William ER Ollier, Robert G Cooper and Neil J McHugh


- 11 / 266 (4.9%) were positive for anti-SAE
- Found exclusively in 8.4 % of adult DM patients
- Specific clinical features
- Strong association with HLA DRB1*04/ DQB1*03
Case D male born 1953

• Acute admission RUH March 2014
  • PUO
  • 4/12 fatigue, muscle aching and weakness, weight loss
  • Worsening anaemia Hb 85
  • CRP 90, PV 2.71, normal myeloma screen, CK, CEA, CA19.9
  • Normal CT scans, colonoscopy and temporal artery biopsy
  • MR thighs – muscle atrophy
  • Positive anti-TIF-1γ autoantibody

• PMHx
  • Type 2 diabetes
  • Renal cell carcinoma in 2011
    • Nephrectomy (SOURCE RCT Sorafenib vs placebo)
    • Three monthly follow-up in remission
Case D

- May 2014
  - Partial response but relapse on pred 40 mg/day
  - Bibasal lung crackles and TLCO 64%
  - Proximal muscle wasting
  - Proceed to cyclophosphamide pulses
  - Request PET-CT scan
- PET/CT scan
  - Avid left mid-abdomen node and adjacent thickened bowel
- Laparoscopic biopsy – metastatic renal cell carcinoma
- July 2015
  - Removal of lesion has led to a sustained recovery
Cancer associated myositis

• CAM mostly in DM with incidence ratio 2.4 – 7.7
  • Ovary, lung, GI tract, breast and nasopharyngeal
  • Presence of anti-TIF1γ
    • specificity 89%
    • sensitivity 70%
    • negative predictive value 93%
    • diagnostic odds ratio 18
      • Selva-O’Callaghan Curr Opin Rheum 2010
Learning points from Case C

• Presence of anti-TIF-1γ in adult DM requires very careful screening strategy for occult malignancy that may need repeating

• Potential for full recovery with successful treatment of malignancy
Anti-Tif1-γ (p155/140)

Originally described by Targoff et al and Kaji et al in two separate studies

Targets Transcription Intermediary Factor 1

- 155 kDa gamma subunit
- 140 kDa alpha subunit
- Beta subunit (~100kDa) targeted in some patients

Protein involved in cellular differentiation

Found in adult myositis and JDM

- 20% Adult DM
- Up to 36% Juvenile DM

References:

Clinical Associations of TIF1 in EuMyoNet (first 1616 cases – unpublished)

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>TIF1 Negative</th>
<th>TIF1 Positive</th>
<th>p value</th>
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<tbody>
<tr>
<td>Gottrons</td>
<td>29.7%</td>
<td>79.3%</td>
<td>&lt;0.0001</td>
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<tr>
<td>Heliotrope Rash</td>
<td>29.3%</td>
<td>77.3%</td>
<td>&lt;0.0001</td>
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<tr>
<td>ILD</td>
<td>31.2%</td>
<td>16.0%</td>
<td>=0.0038</td>
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<tr>
<td>Cancer (ever)</td>
<td>8.0%</td>
<td>32.2%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CAM</td>
<td>2.3%</td>
<td>20.5%</td>
<td>&lt;0.0001</td>
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<tr>
<td>CADM</td>
<td>0.8%</td>
<td>5.2%</td>
<td>=0.0028</td>
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</tbody>
</table>
Case D male born 1964

- August 2012
  - 10 years left leg pain
  - 3 months cramping hands and calf pain
  - Episodic mild weakness
  - CK 973
  - Normal EMG
  - MRI increase signal left gastrocnemius
  - Muscle biopsy IBM
  - Presence of anti-Mup44
Learning points from Case D

• Anti-Mup44 (cytosolic 5’nucleotidase 1A - cN1A) found in 30-40% of patients with IBM
• May also be found less frequently in other form of autoimmune connective tissue disease
• May prove to be a valuable diagnostic marker
• Discovered as muscle tissue itself was used as a source of antigen
Case E female born 1942

- August 2013
  - 4 weeks progressive proximal muscle weakness legs more than arms
  - MMDS 23/33
  - Statin stopped 3 weeks ago
  - CK 9375, ALT 179
  - Anti-HMGCoAR strongly positive
  - MR atrophy and oedema in thigh muscles
  - Muscle biopsy necrosis and regeneration

- Dec 2013
  - Slow recovery following corticosteroids so IV cyclophosphamide MMDS 20/33

- Feb 2014
  - Improving strength, MMDS 30, CK 178

- March 2015
  - Reaction to azathioprine, CK 49, prednisolone 5 mg/day

- June 2015
  - Well. No muscle weakness. Off treatment CK normal
Learning points from Case E

• Statin-induced myositis may be associated with antibodies to HMGCoAR
• Full recovery in this case with discontinuation of statin
• Levels of anti-HMGCoAR may help in monitoring disease
• Anti-HMGCoAR have also be found in cases with no history of statin use
Autoantibodies in Myositis

• Identify distinct subsets of disease that differ in frequency between adults and children
  • Clinical
  • Genetic
  • Environmental
• Help give insight into the cause of disease
• Have become a highly useful in diagnosis and predicting outcome so informing treatment decisions and may help avoid more invasive investigations
• The actual level of the autoantibody may reflect the amount of active disease and therefore help guide treatment
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