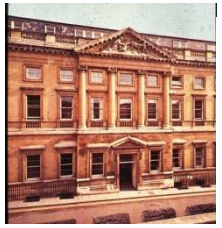
UNIVERSITY OF BATH



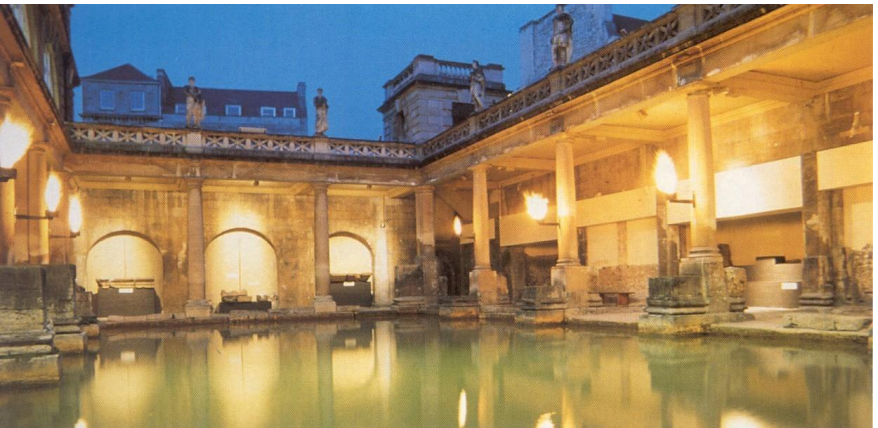
Royal National Hospital for Rheumatic Diseases
NHS Foundation Trust

What are Autoantibodies and how do they work in Myositis?

Neil McHugh, University of Bath and

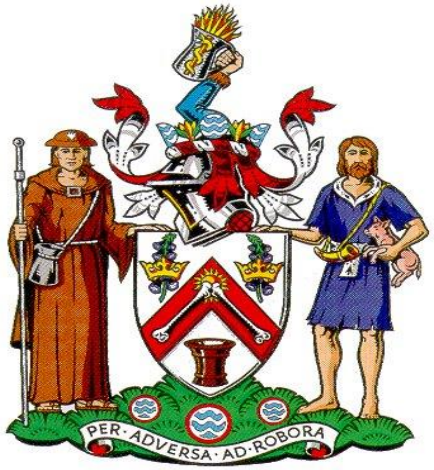
Royal National Hospital for Rheumatic Diseases

Orlando September 2015

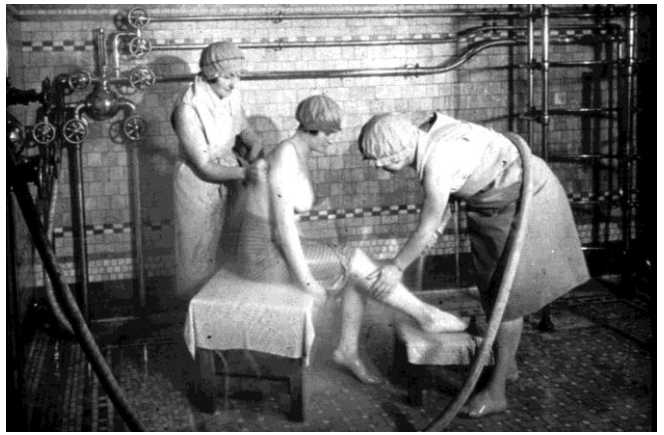


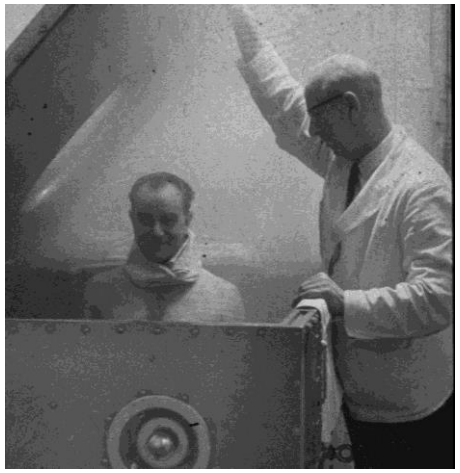
THE MYOSITIS ASSOCIATION

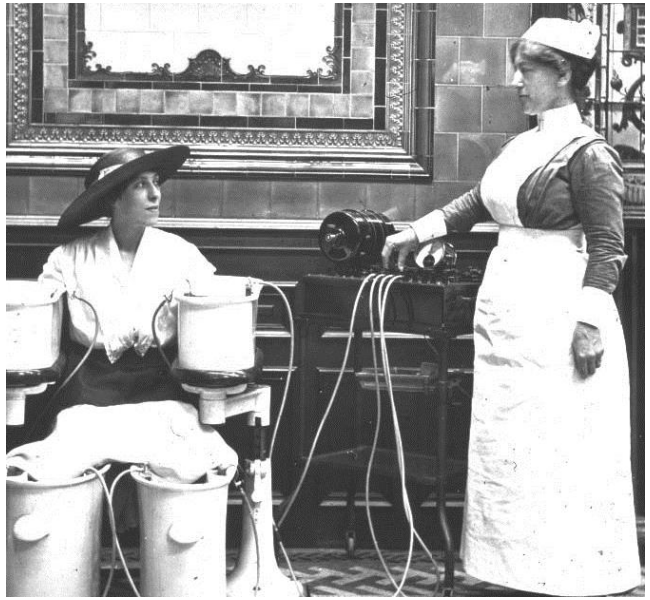
Royal National Hospital for Rheumatic Diseases
Founded in 1738



Historical treatments for Arthritis



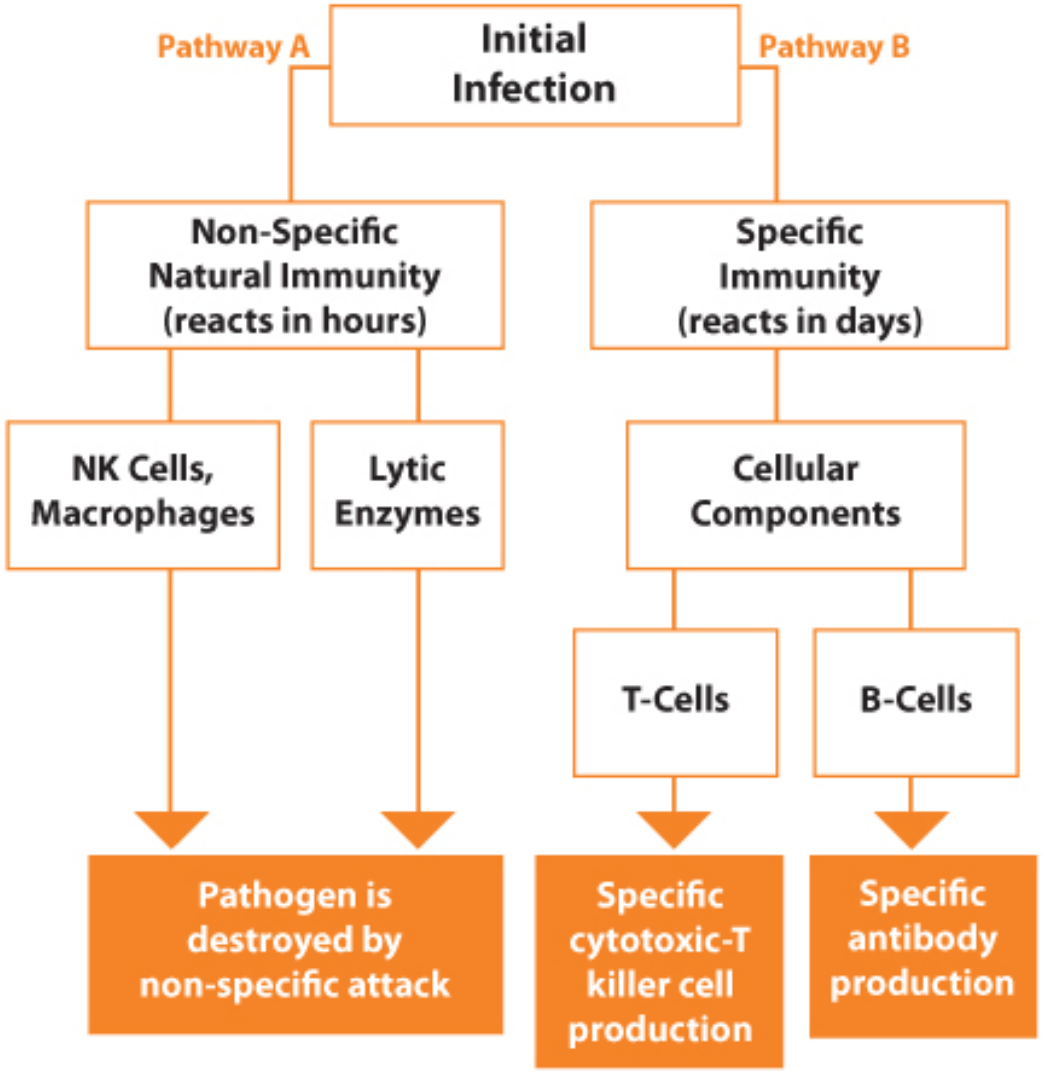




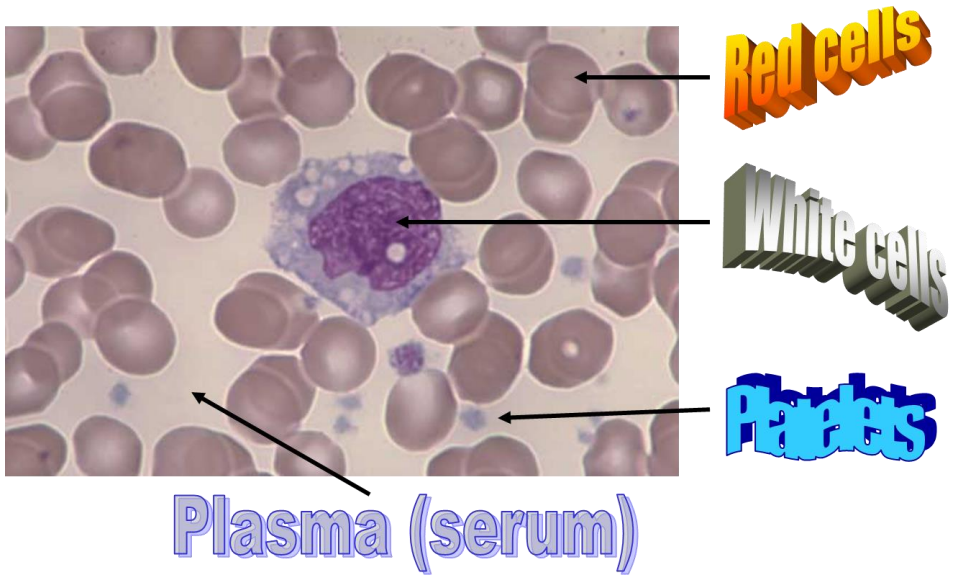
What are Antibodies?

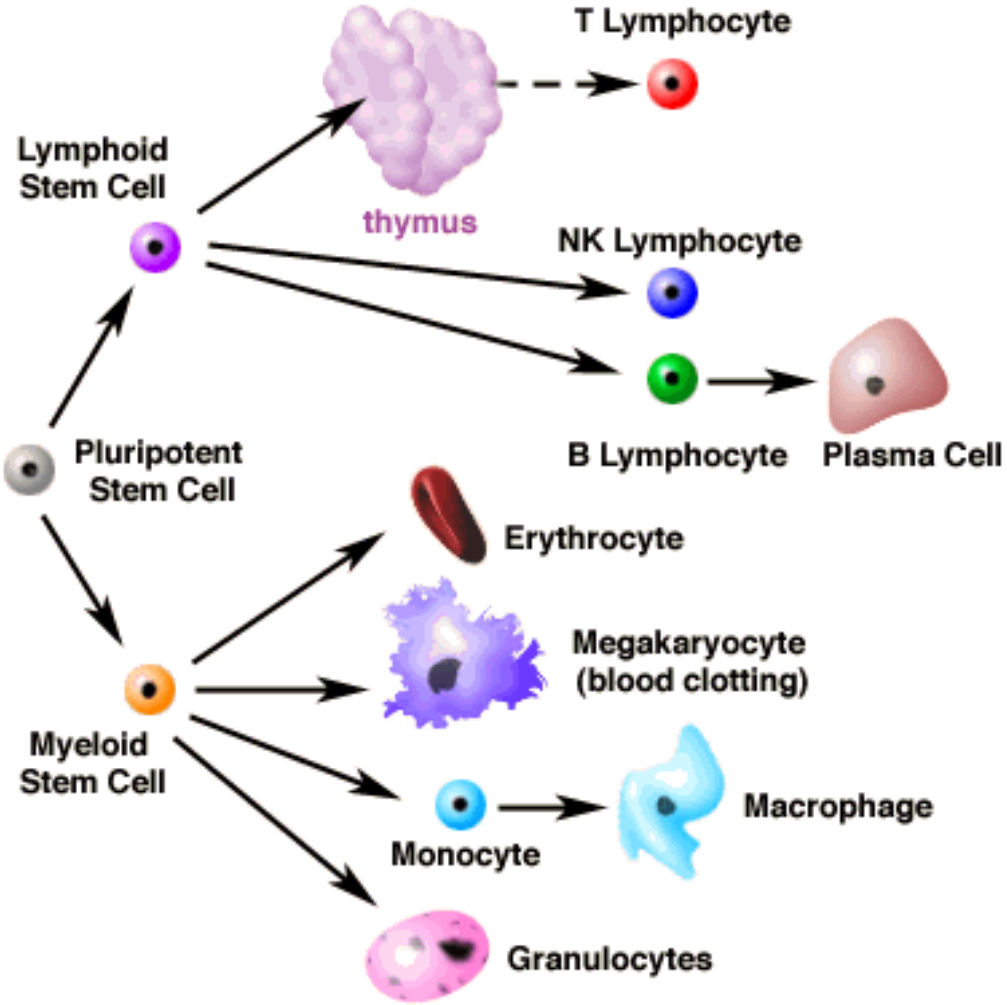
What are Autoantibodies?

Antibodies are part of the ‘immune system’

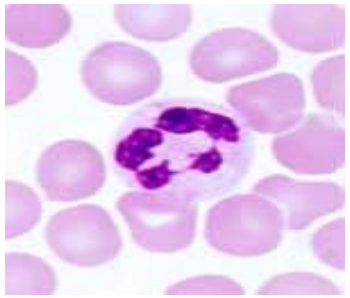


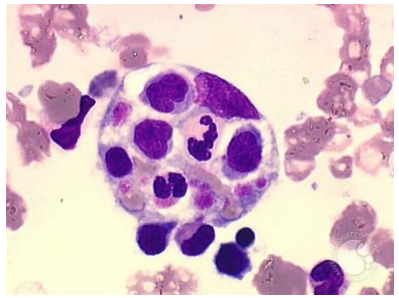
Types of cells in the blood

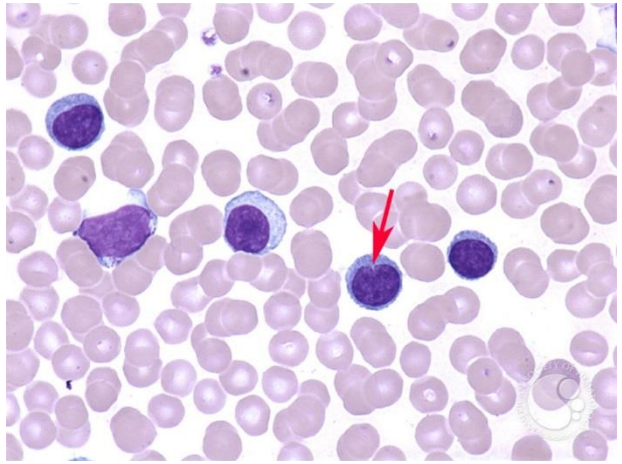


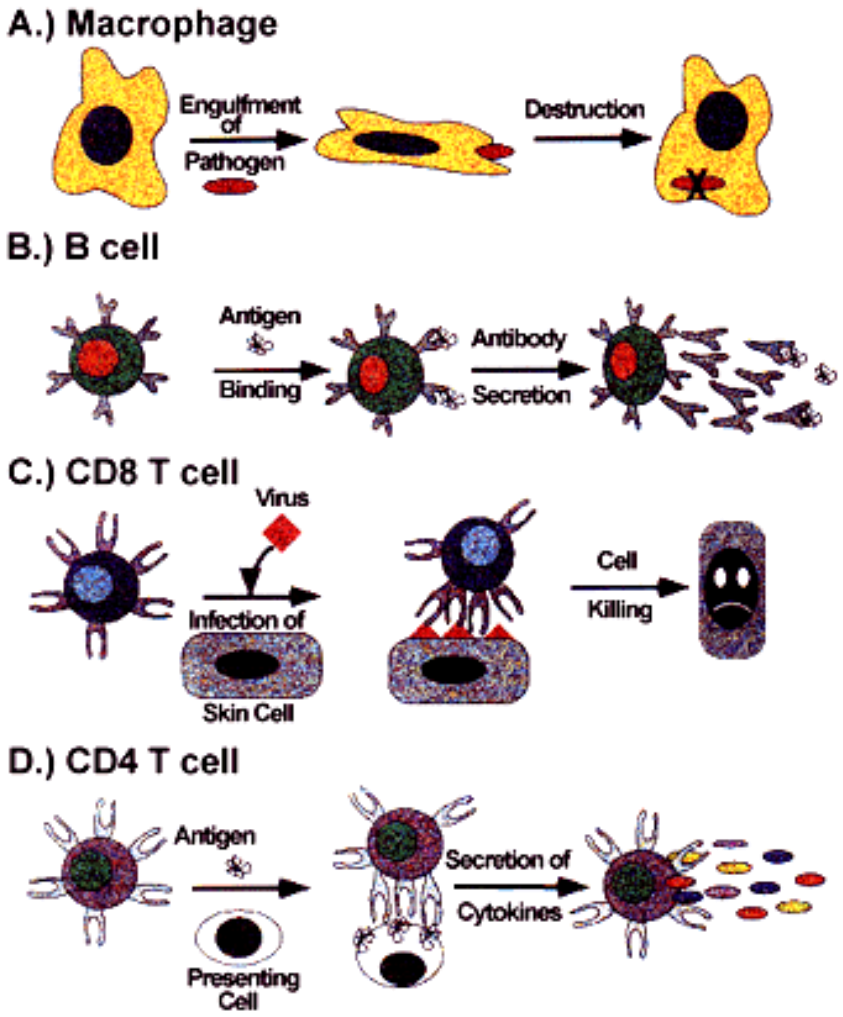


Blood cells that make up the immune system

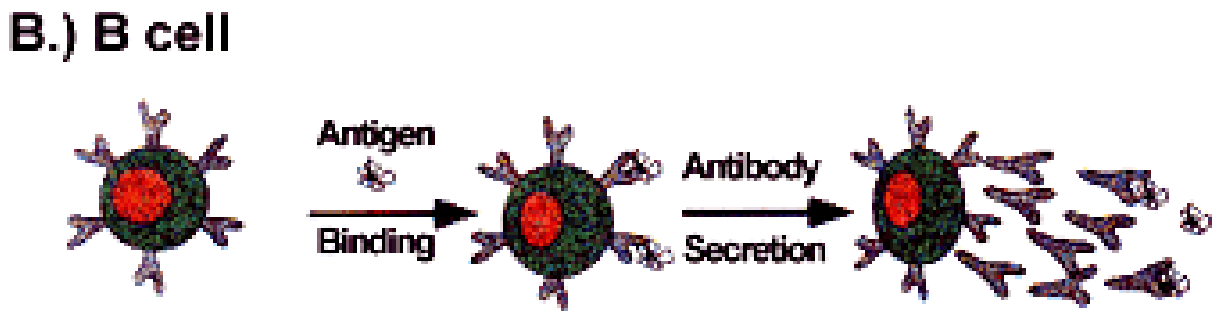


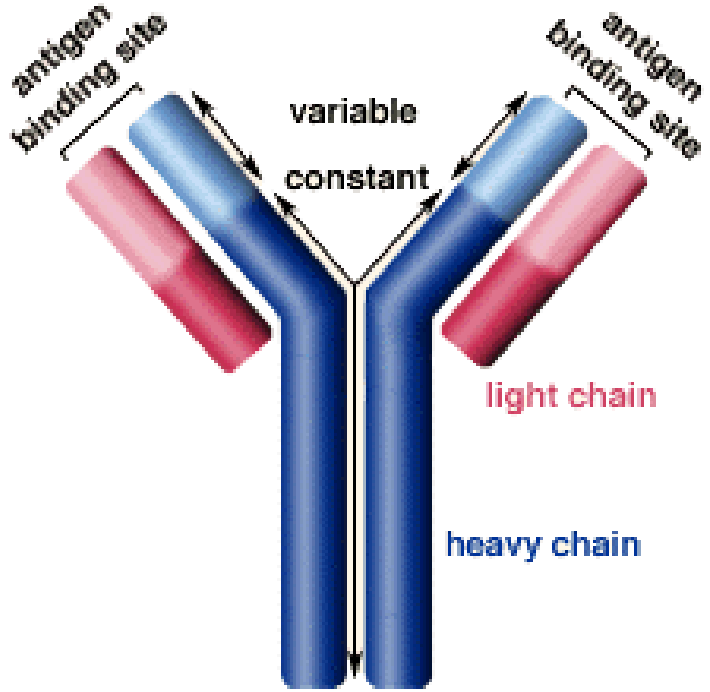




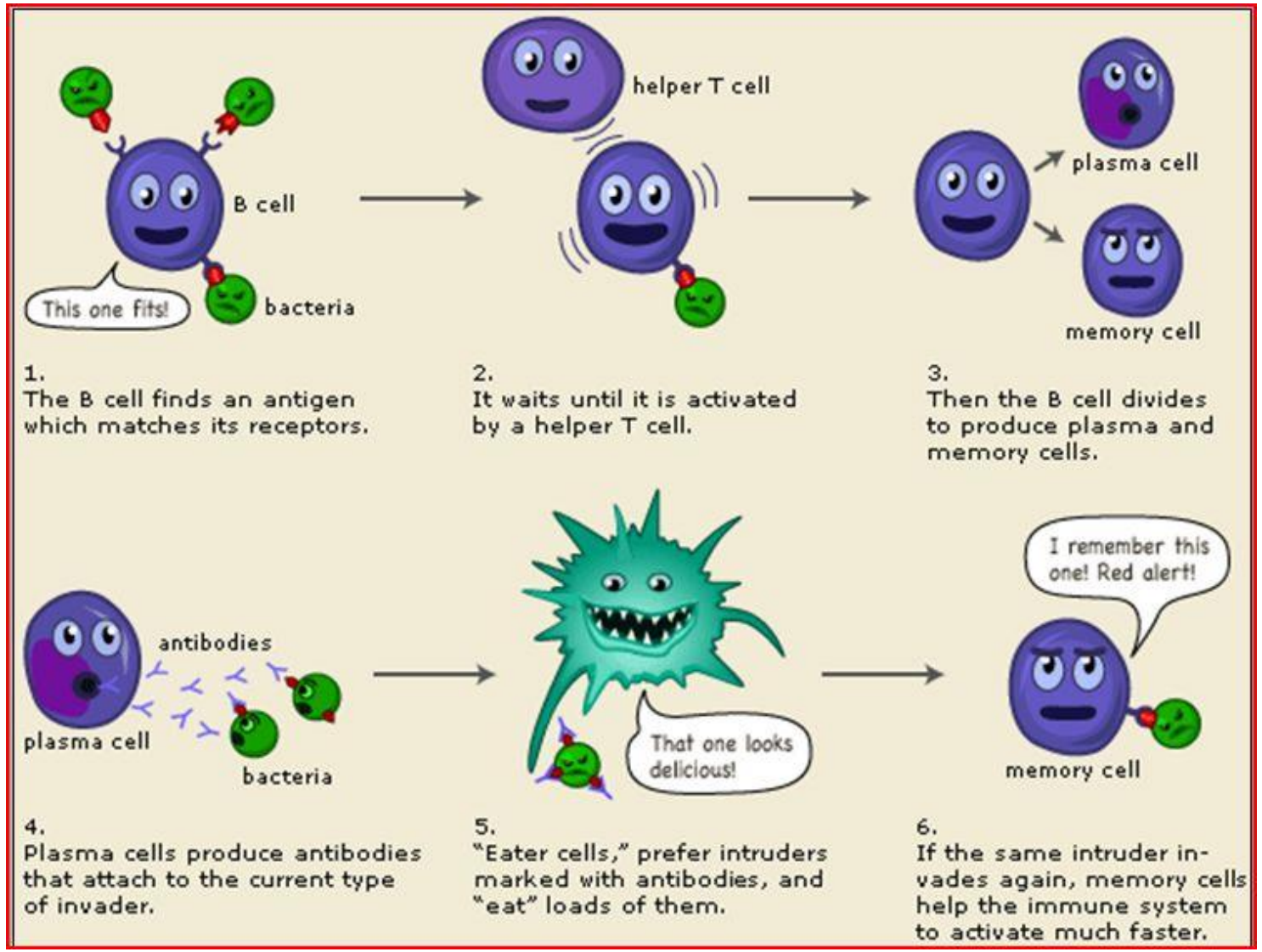


B cells make antibodies (immunoglobulin)





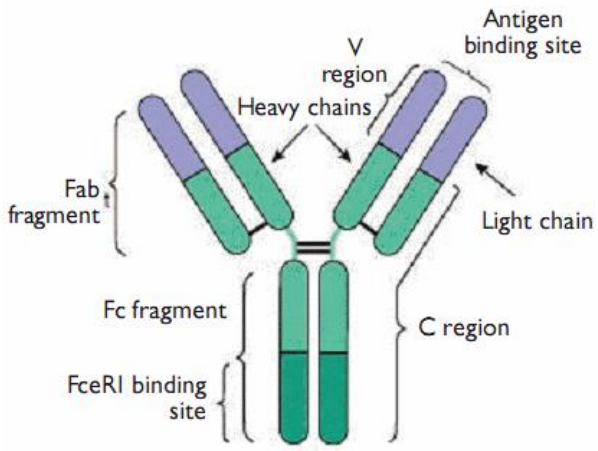
Antibodies provide protection from infection



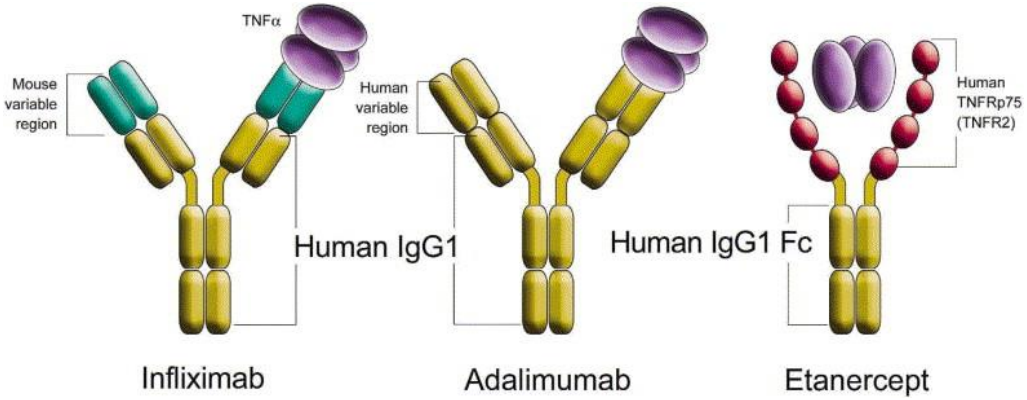
What are Antibodies?

What are Autoantibodies?

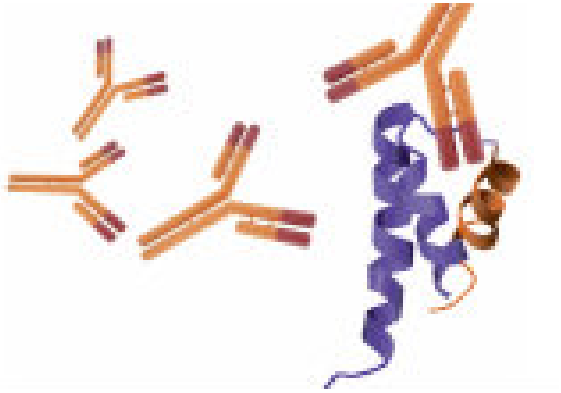
Antibodies and Autoantibodies



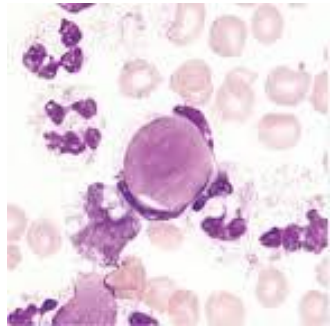
Immunoglobulin



Biological therapies



Autoantibody (anti-DNA)



LE cell first diagnostic test for SLE

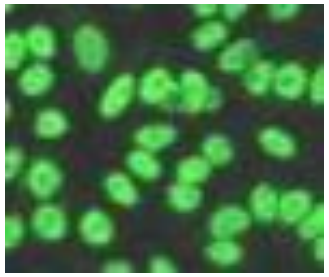
What are autoantibodies?

- **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**

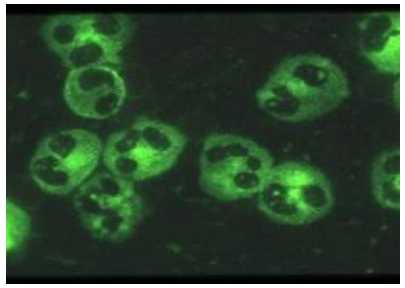
Methods for detecting autoantibodies

Autoantibody Screening by Indirect Immunofluorescence

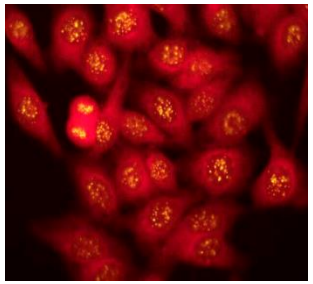
Hep-2



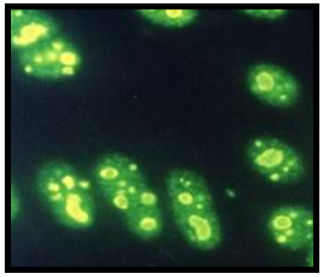
Human neutrophil



Hep-2

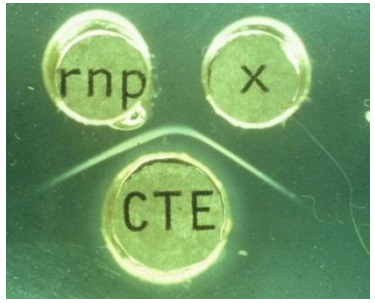


Hep-2



Autoantibody identification by second technique

Immunodiffusion

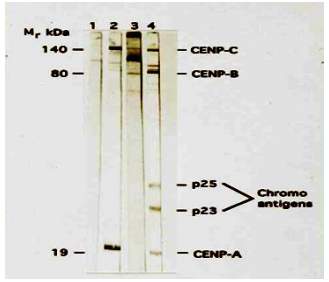


ENA
anti-RNP

ELISA

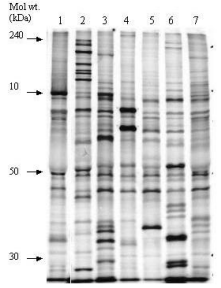
anti-PR3

Immunoblot



anti-centromere

Immunoprecipitation

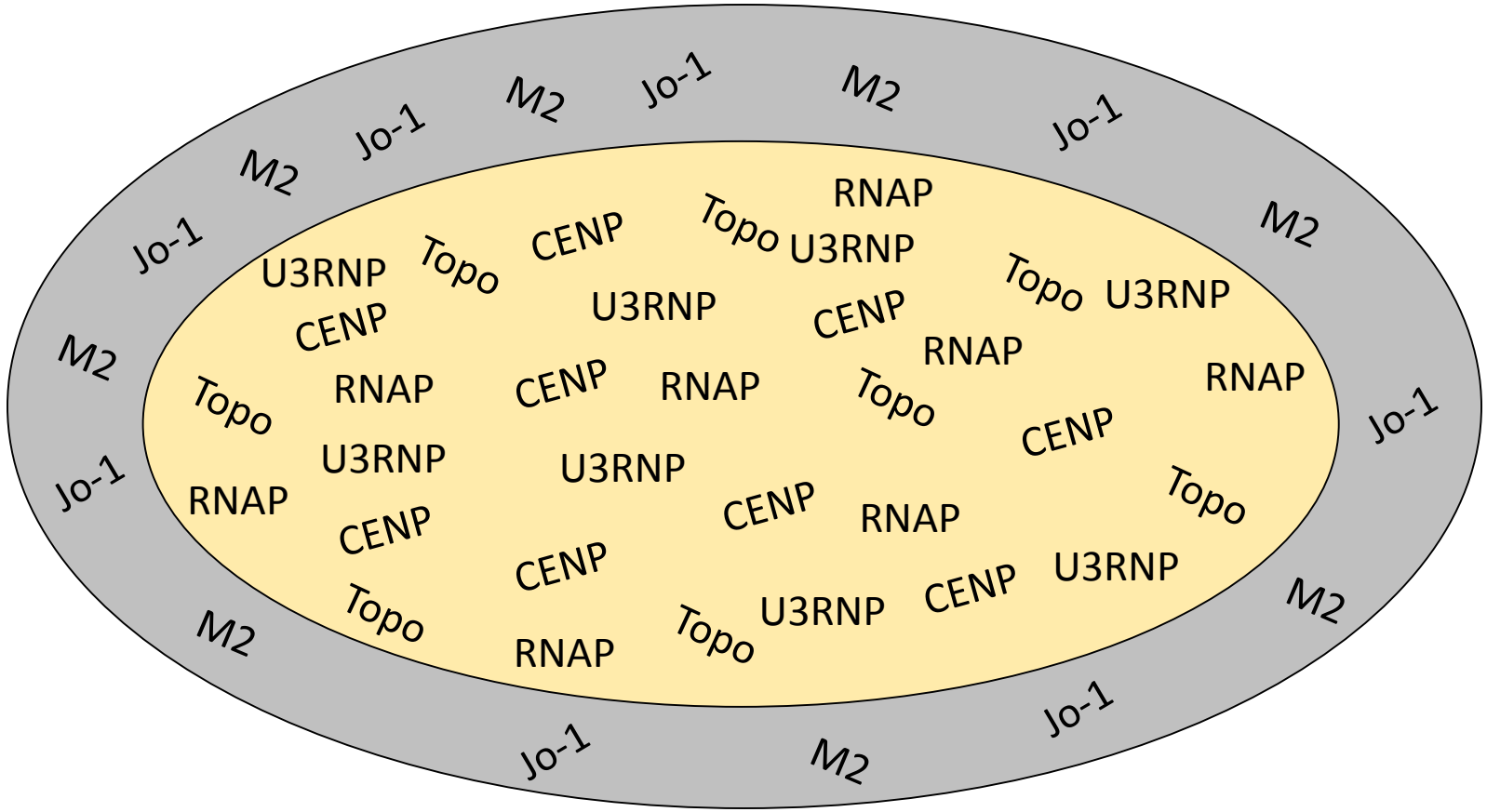


Anti-fibrillarin
U3RNP

Indirect Immunofluorescence

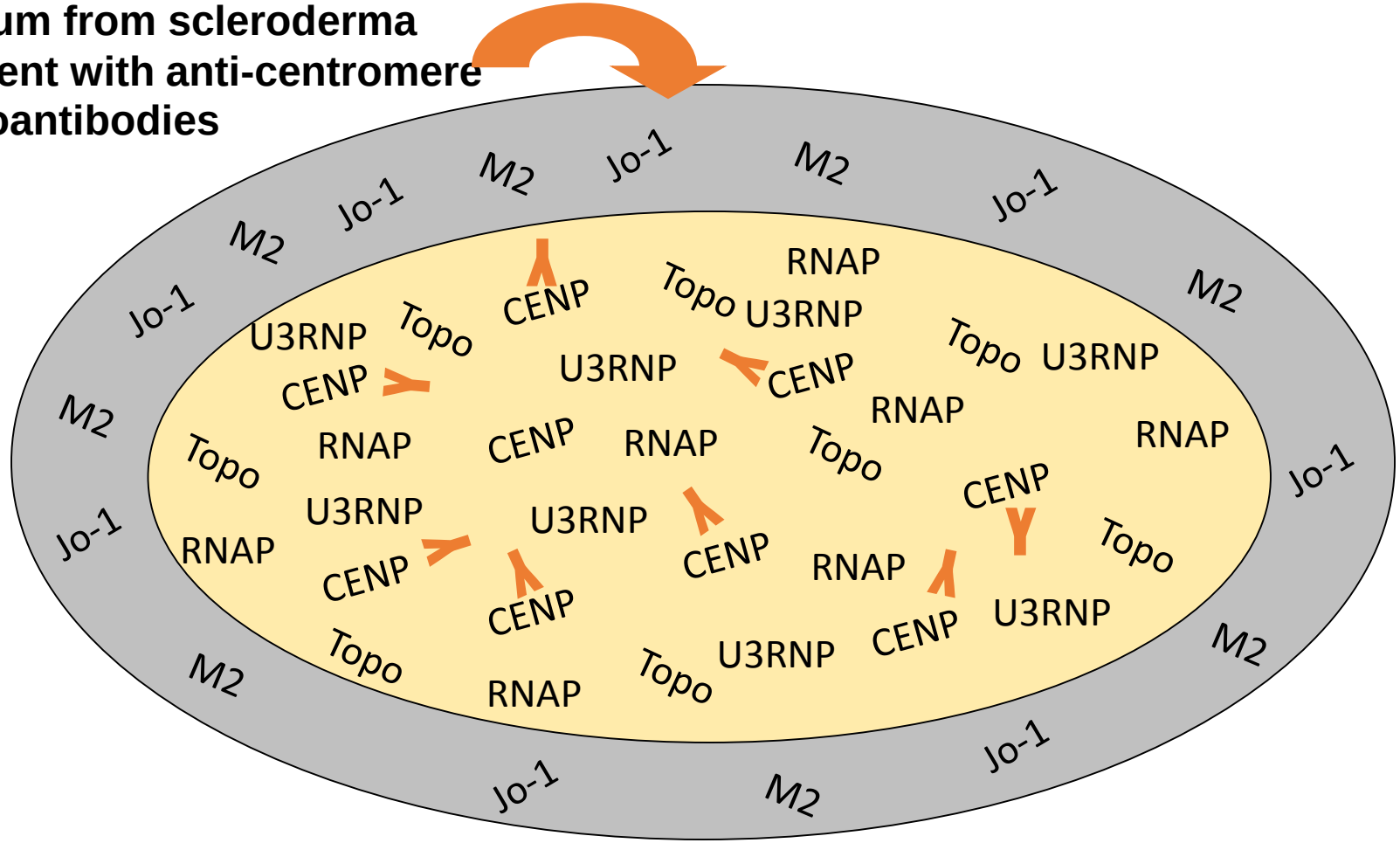
- **Antigen source -** **tissue section (mouse LKS, monkey oesophagus) whole cell (HEp-2, neutrophil, *crithidia luciliae*)**
- **Autoantibody from patient serum - Apply autoantibody that if present will bind to the antigen source**
- **Secondary antibody - anti-human IgG FITC**
- **Visualization - green fluorescence in a recognizable pattern corresponding to location of antigen read under a specialized immunofluorescence microscope**

Indirect immunofluorescence test I

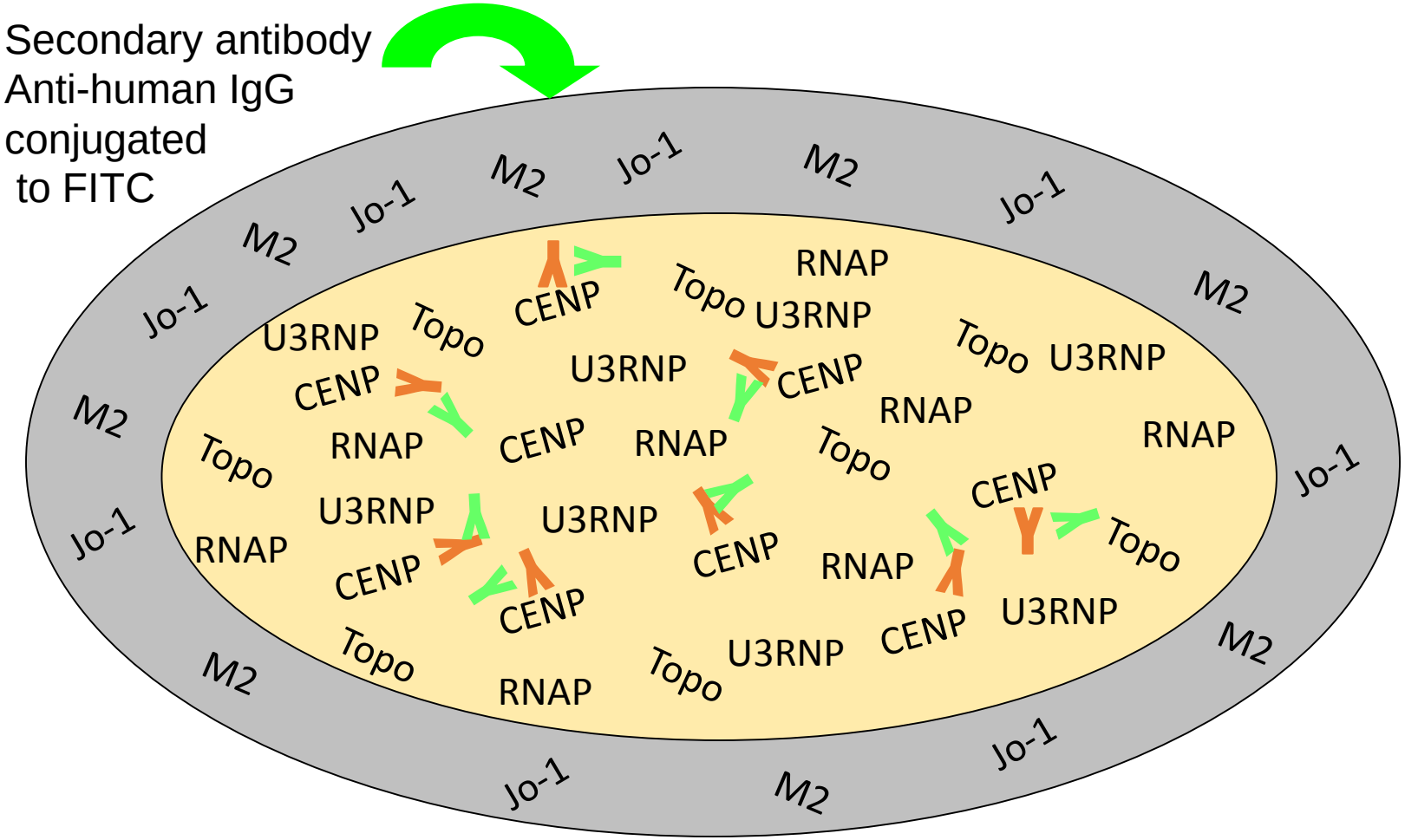


Indirect immunofluorescence test II

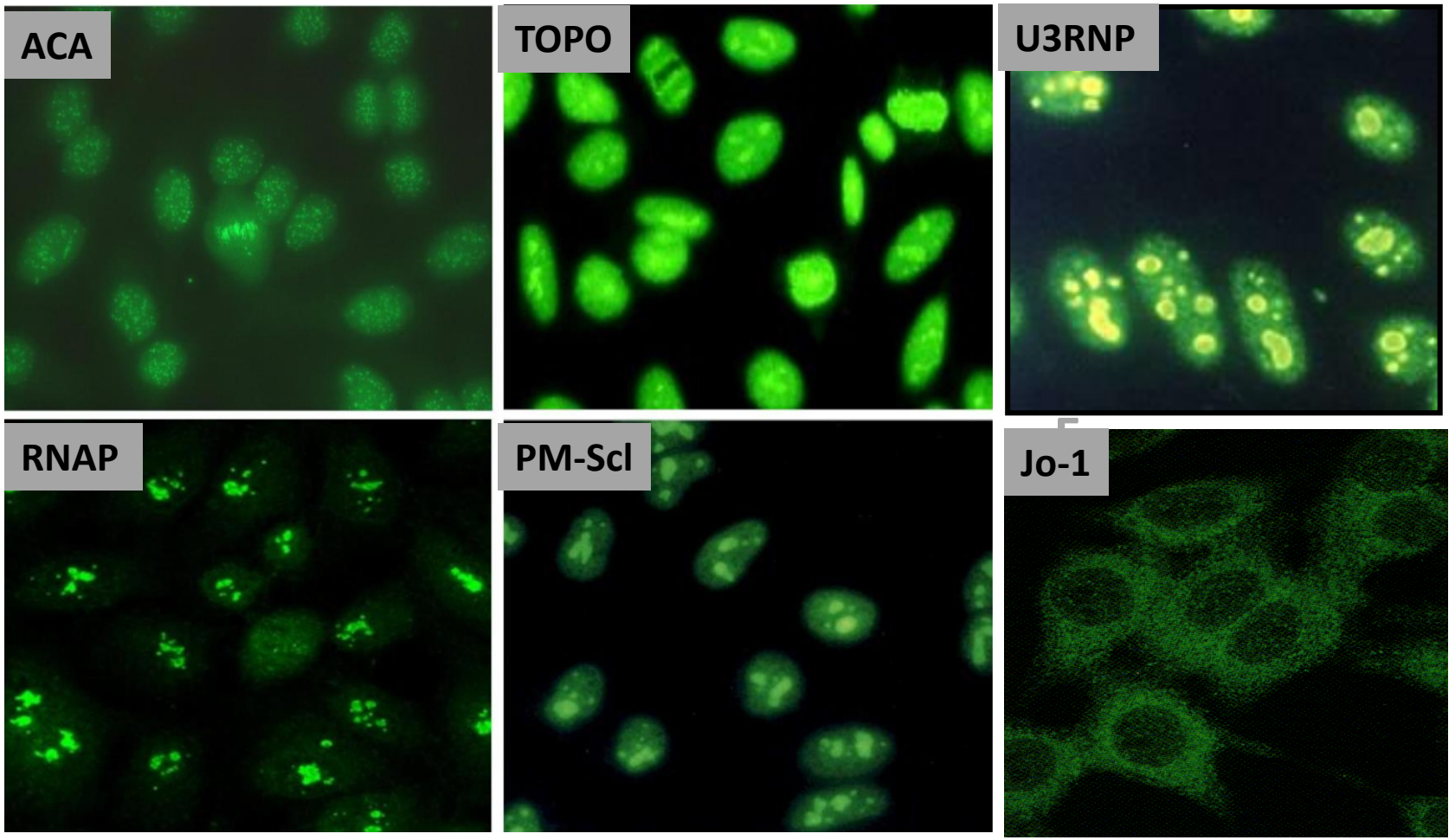
Serum from scleroderma patient with anti-centromere autoantibodies



Indirect Immunofluorescence test III

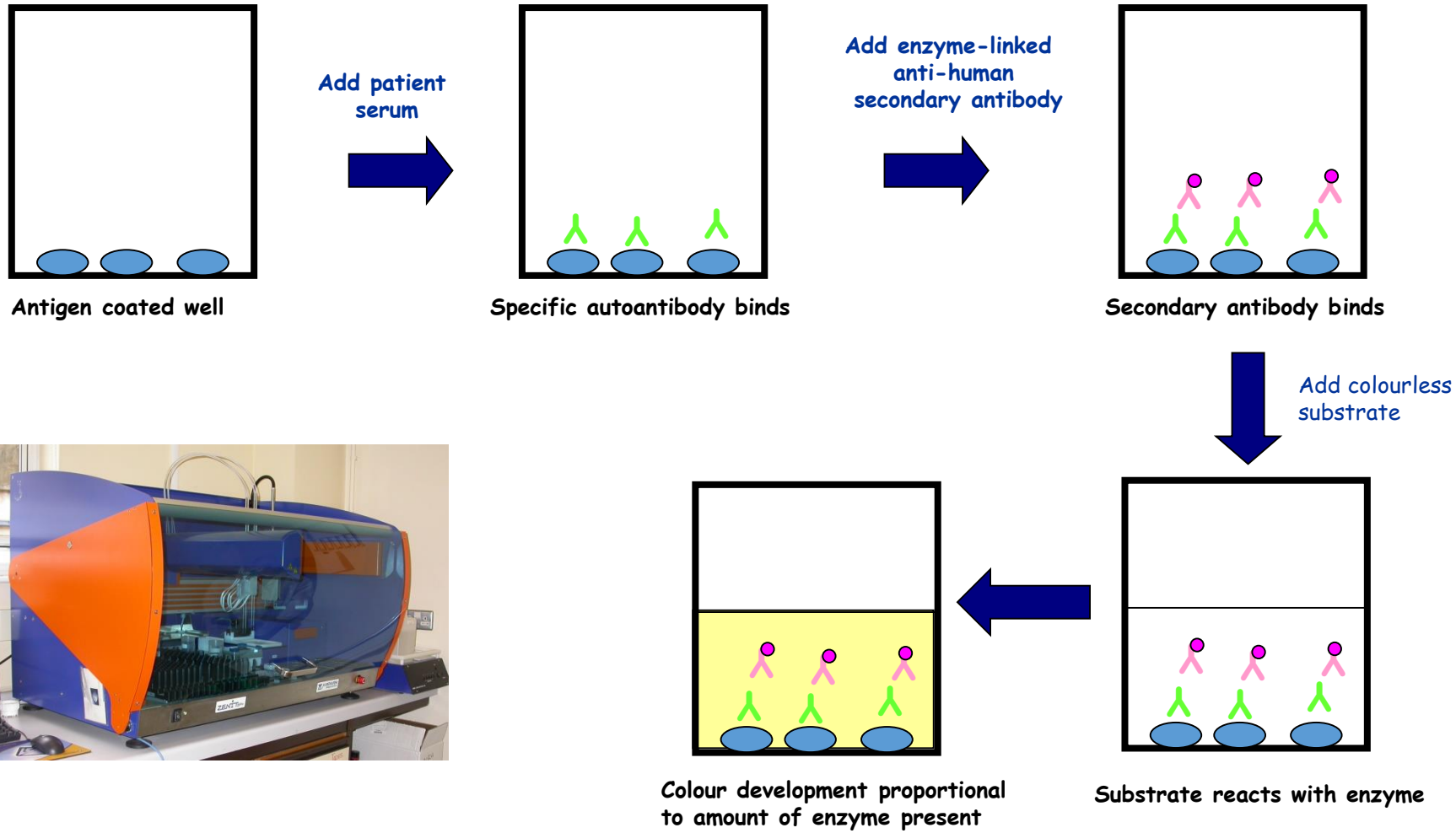


Indirect immunofluorescence

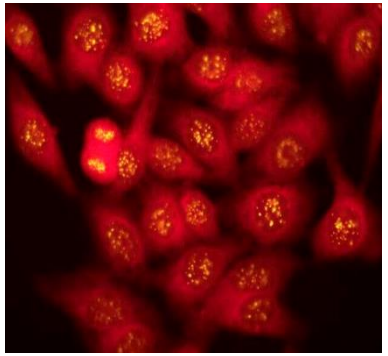


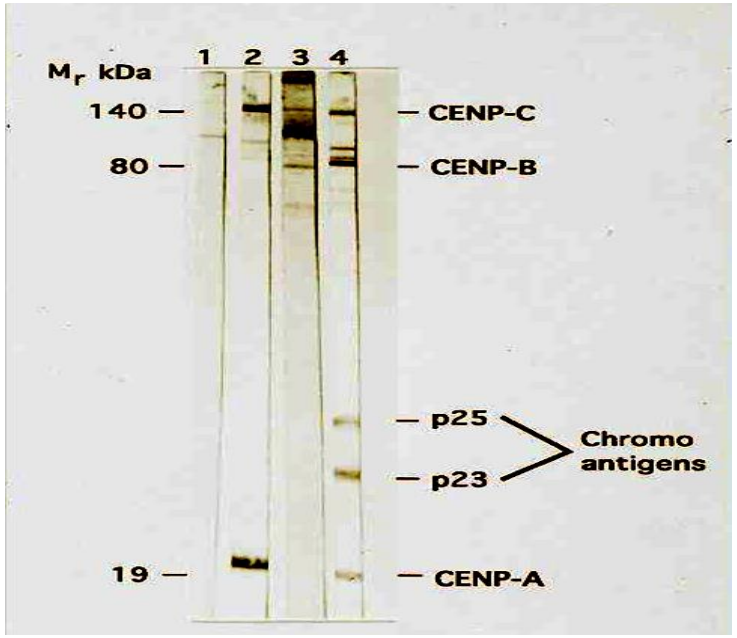
- If test positive the patient will be reported as having an antinuclear antibody (ANA)
- Sometimes the pattern will reveal the type of ANA (specificity) but usually another method will be necessary for exact identity

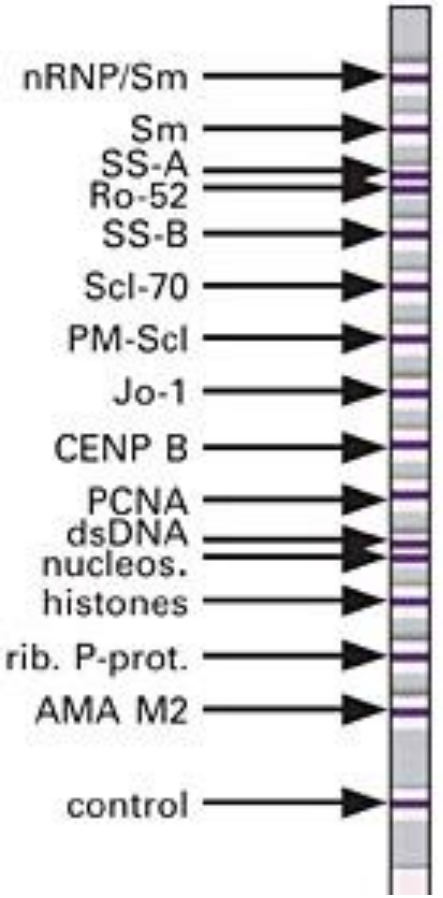
Enzyme-linked immunosorbent assay (ELISA)



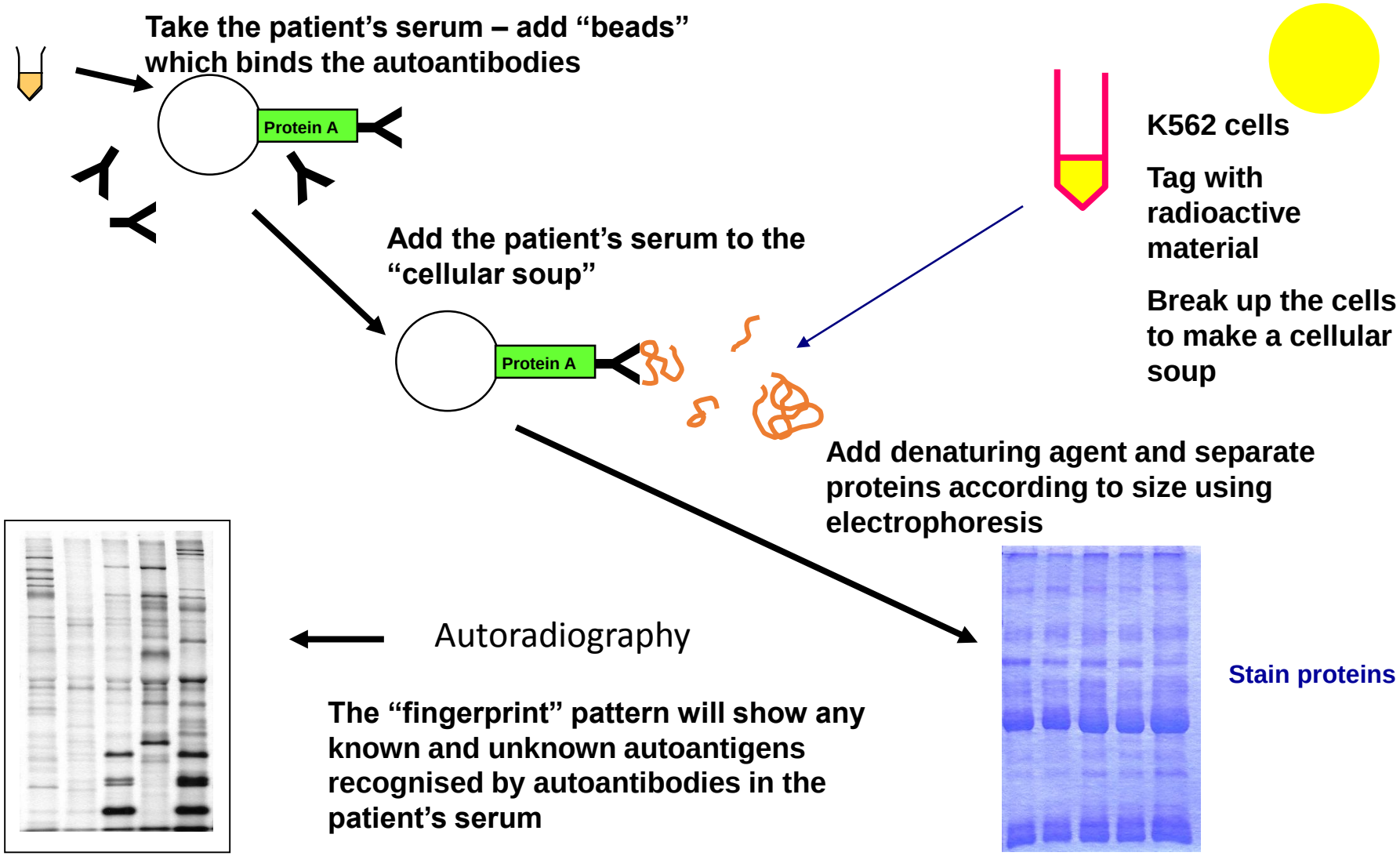
Immunoblot and Lineblot



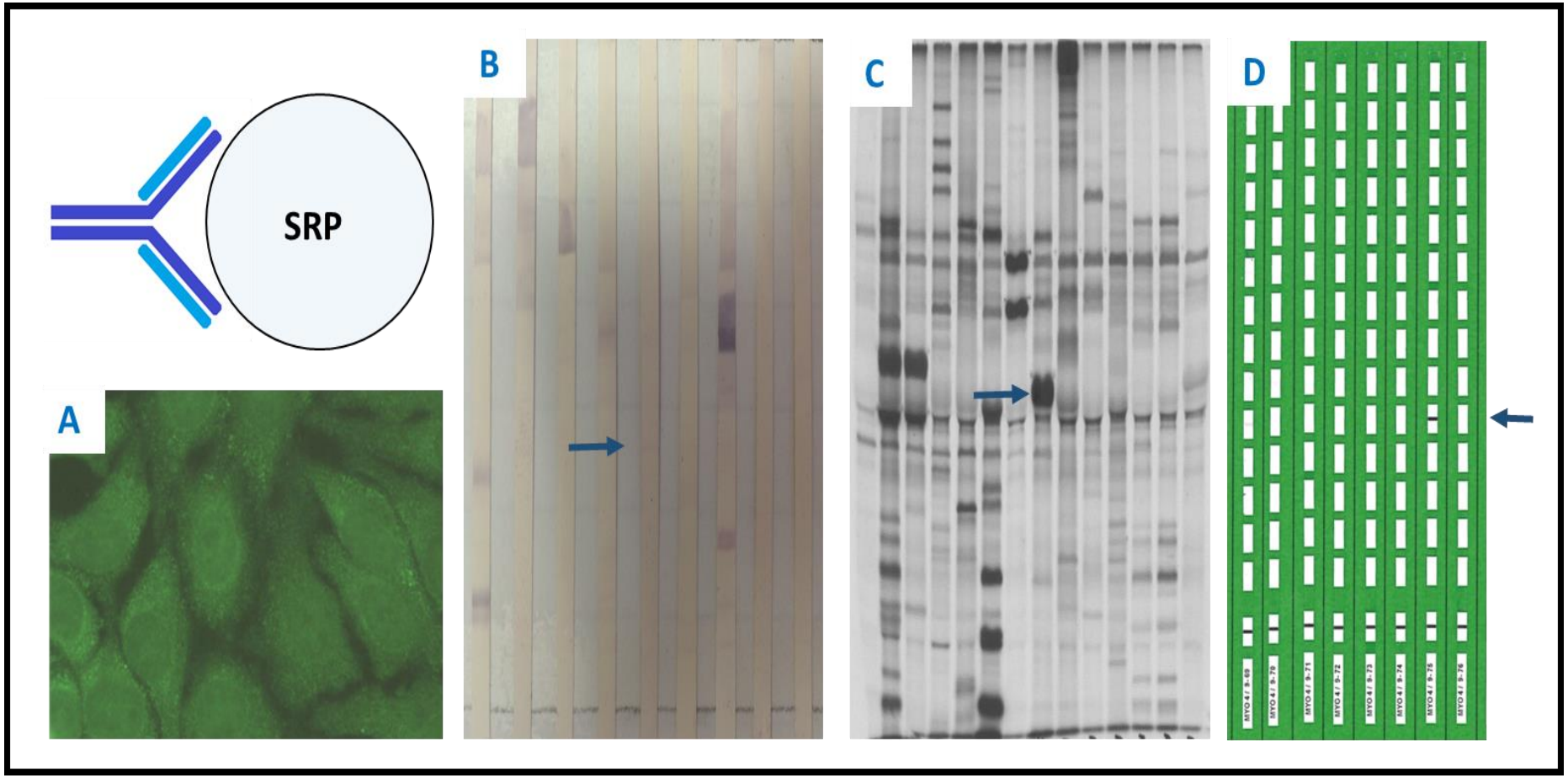




Autoantibody detection by protein immunoprecipitation

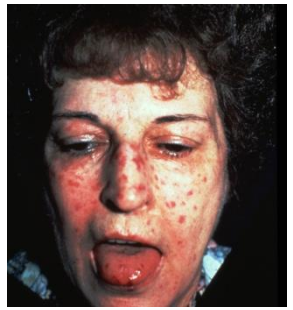


Anti-signal recognition particle (SRP) by different assays



So what does all this mean?

The spectrum of autoimmune connective tissue disease

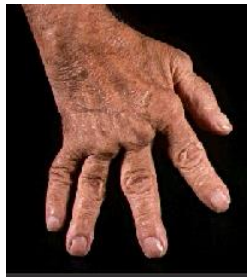


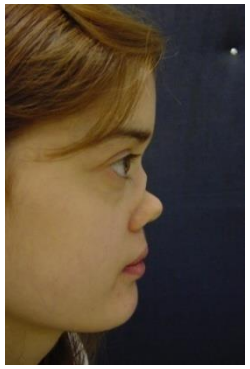
Scleroderma

Nucleolar RNP

Rheumatoid arthritis

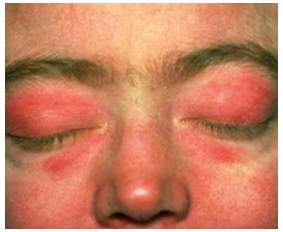
ACPA





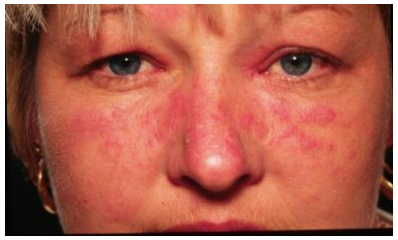
Granulomatosus with polyangiitis

ANCA



Dermatomyositis

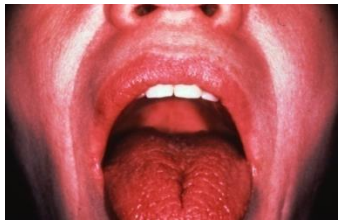
Transcription tactors RNA synthetase



Systemic Lupus Erythematosus

snRNPS

Nucleosome

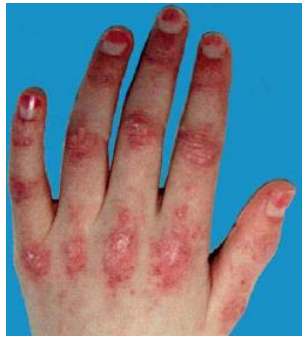
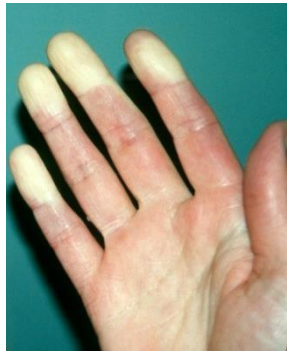
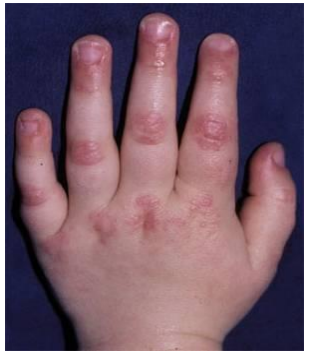
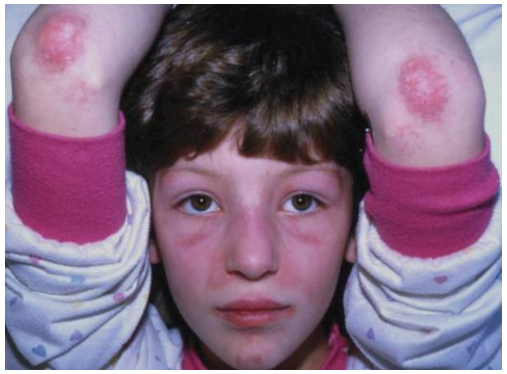
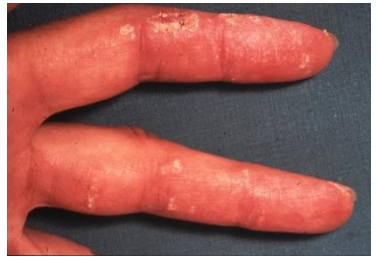
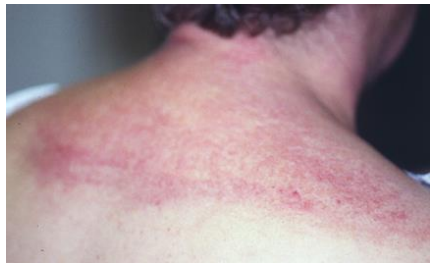
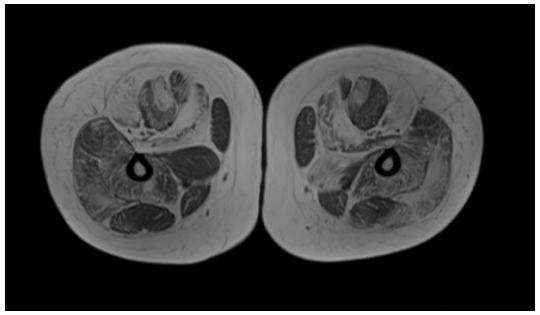
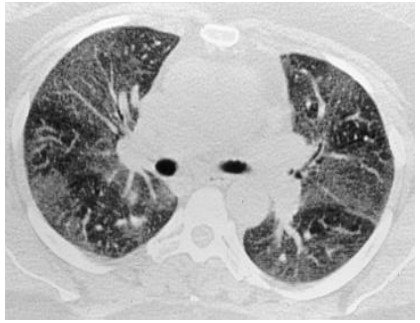
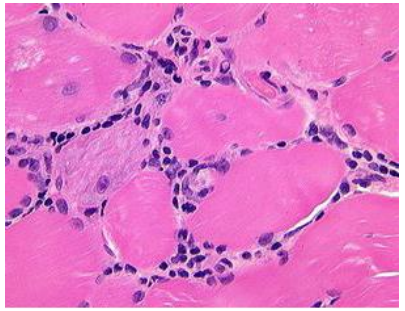
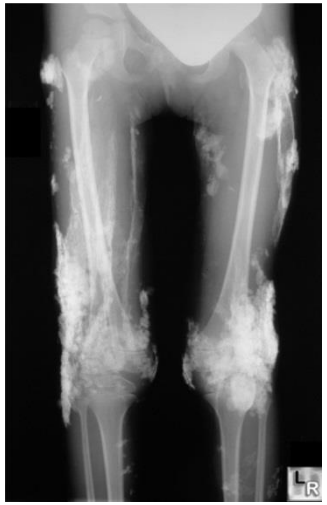


Sjogren's

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

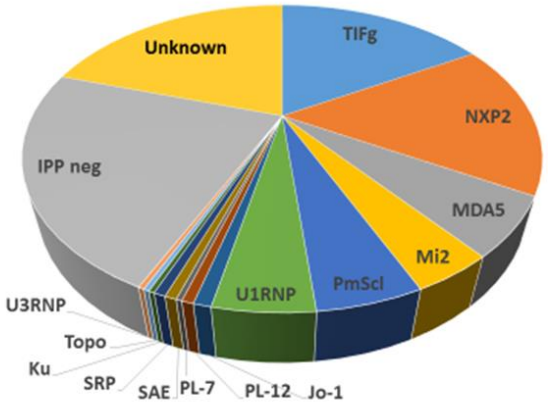
Autoantibodies in myositis

- 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

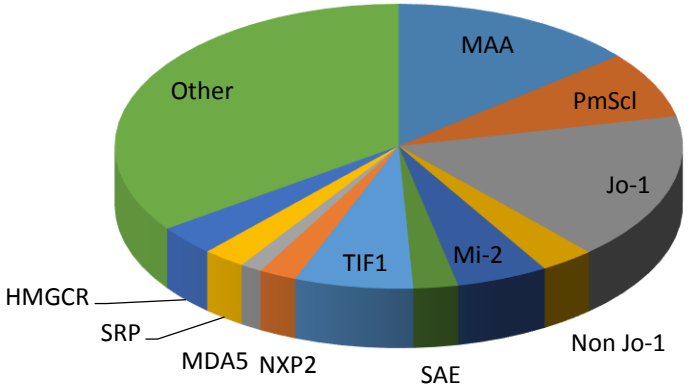
Patterns of juvenile versus adult myositis

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



UK JDM Cohort and Biomarker Study n= 347

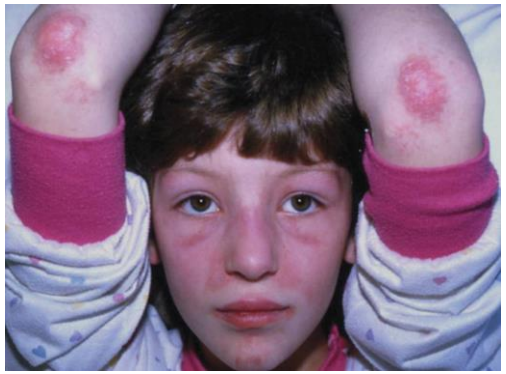
- Adult myositis
  - Dermatomyositis
    - Association with malignancy
  - Polymyositis
    - Antisynthetase syndrome
  - Inclusion body myositis
  - Overlap



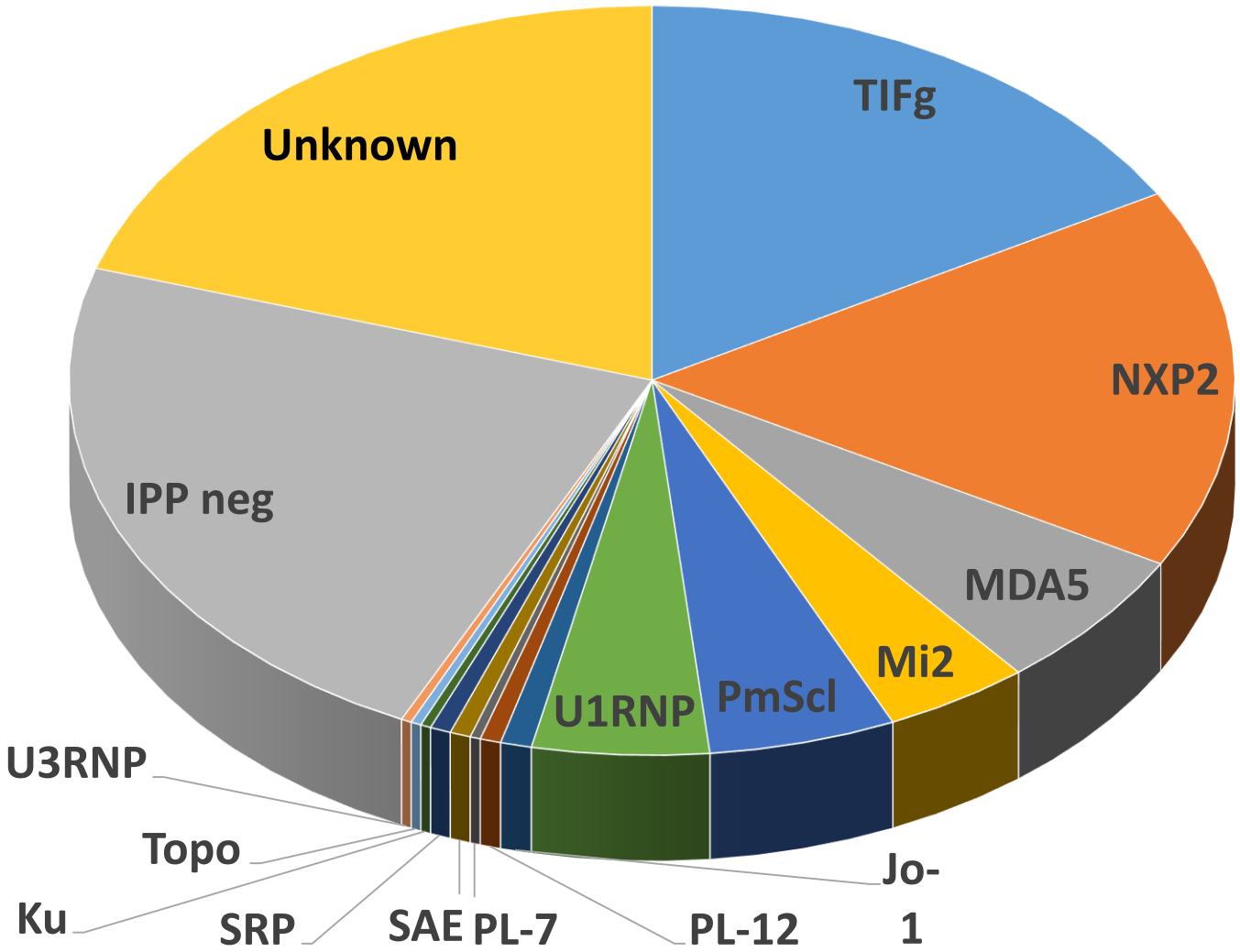
EUMYONET n = 1616

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



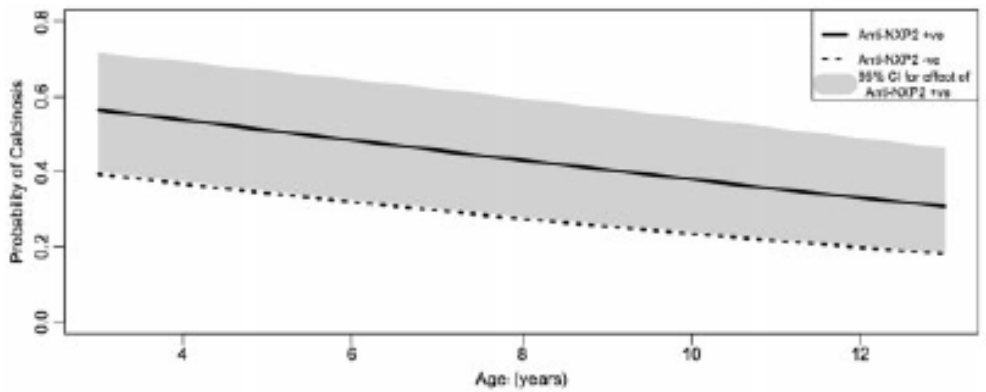
UK JDM Cohort and Biomarker study n = 347



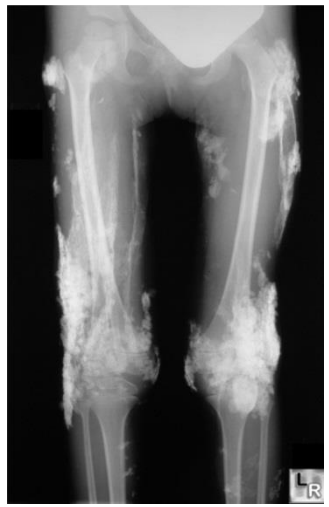
Calcinosis in juvenile dermatomyositis is influenced by both anti-NXP2 autoantibody status and age at disease onset

Sarah L. Tansley[1], Zoe E. Betteridge[2], Gavin Shaddick[3], Harsha Gunawardena[4], Katie Arnold[5,6], Lucy R. Wedderburn[5,6] and Neil J. McHugh[1], 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.



Tansley S *et al.* Rheumatology 2014;53(12):2204-8.

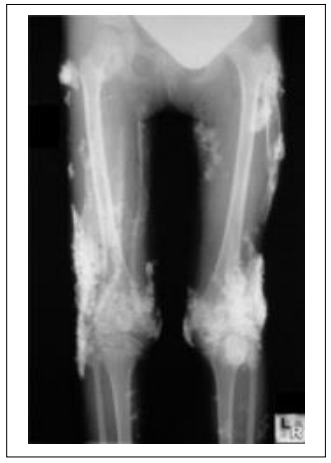
Anti-NXP2 Autoantibodies

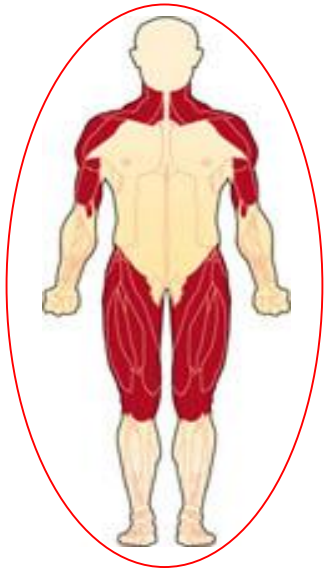
Children **Young Adults** **Older Adults**

AGE

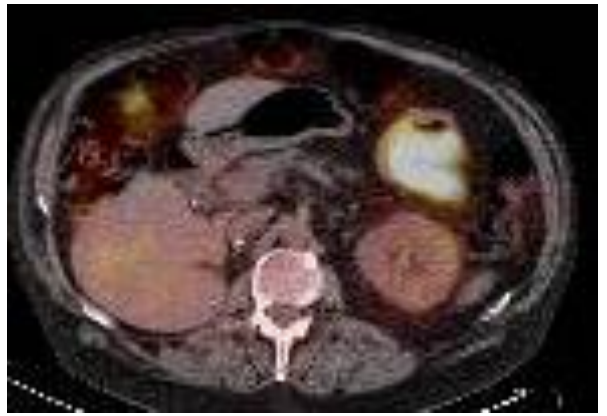
Rash

Calcinosis





Malignancy



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

Sarah L Tansley[1], Zoe E Betteridge[2], Harsha Gunawardena[3], Thomas S Jacques[4], Catherine M Owens[5], Clarissa Pilkington[6], Katie Arnold[7], Shireena Yasin[7], Elena Moraitis[6], Lucy R Wedderburn[8], and Neil J McHugh[9*] on behalf of UK Juvenile Dermatomyositis Research Group

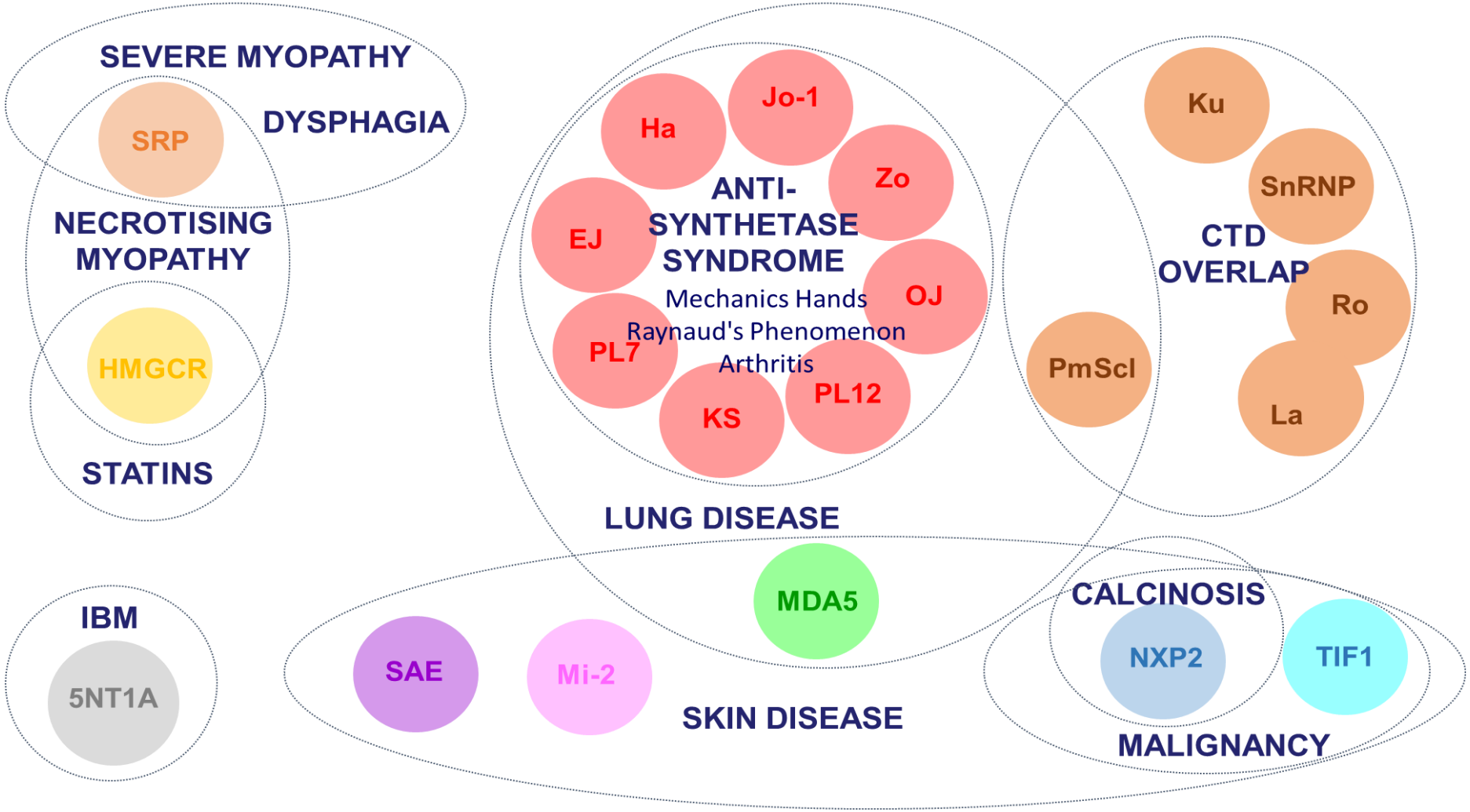
- **Anti-MDA5 in 7.4% of JDM patients**
- **Associated with skin ulceration, oral ulceration and milder muscle disease**
- **Milder muscle biopsy in all four domains of JDM biopsy score**
- **4 of 21 had interstitial lung disease**

Arthritis Res Ther 2014

Summary of autoantibodies in JDM

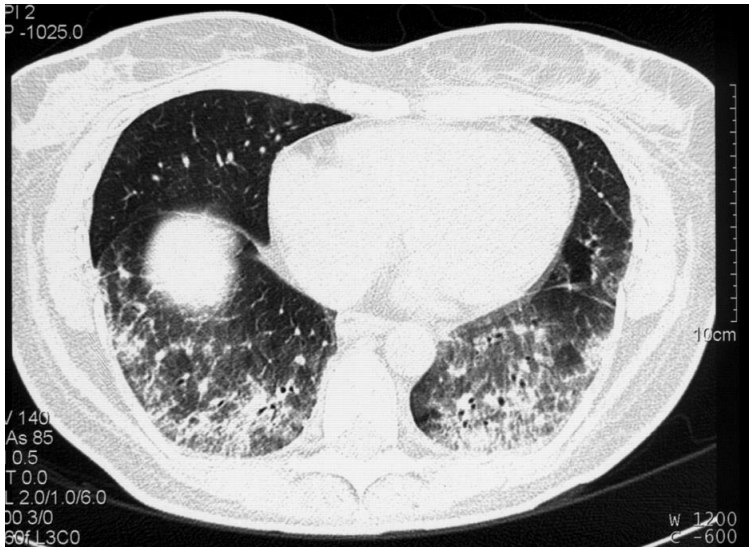
- Myositis autoantibodies (MSA and MAA) in JDM
  - Present in 60% of cases
  - May be valuable in diagnosis
  - Newer specificities (TIF1, NXP2, MDA5) account for 40% of cases and identify clinical subsets of disease
  - Have different prevalence and associations across the myositis spectrum dependent 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



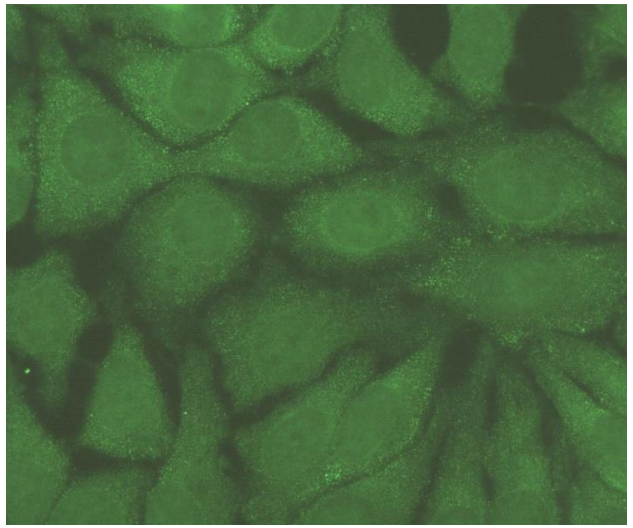
Case A female born 1957

- **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**



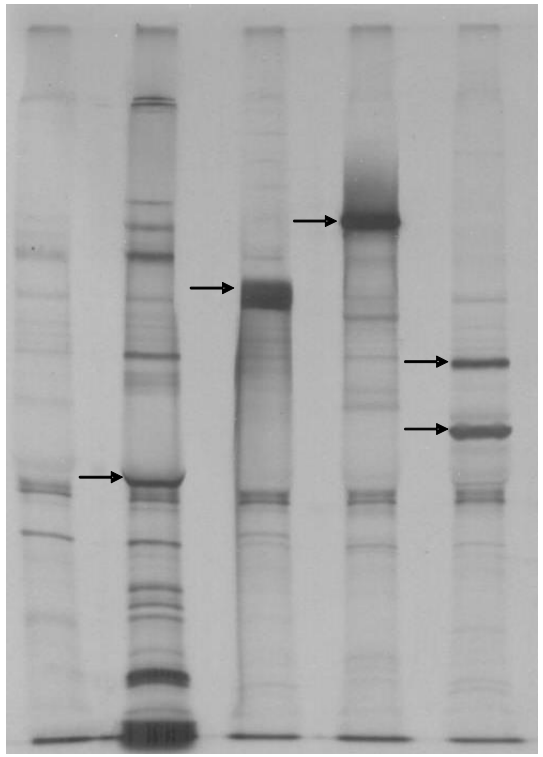
Anti-synthetase syndrome: a new autoantibody to phenylalanyl transfer RNA synthetase (anti-Zo) associated with polymyositis and interstitial pneumonia

Z. Betteridge[1], H. Gunawardena[1,2], J. North[1], J. Slinn[3] and N. McHugh[1,2]



Strong Cytoplasmic Speckle on Indirect Immunofluoresence

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

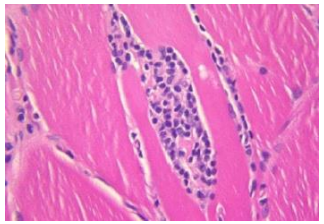


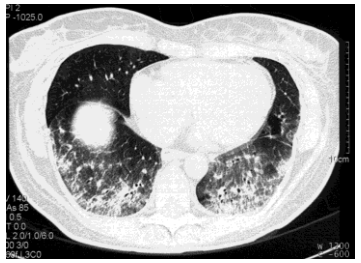
Protein Immunoprecipitation of bands at approximately 60 kDa and 70 kDa – phenylalanyl tRNA synthetase

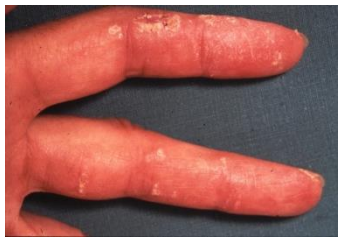
Anti-synthetase syndrome

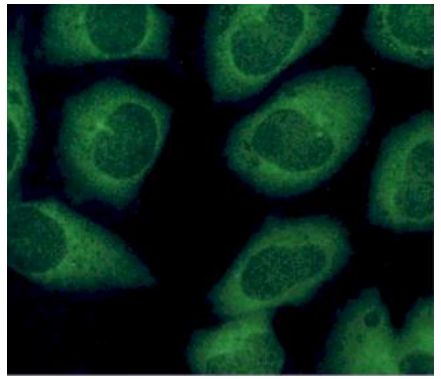
Clinical Features

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

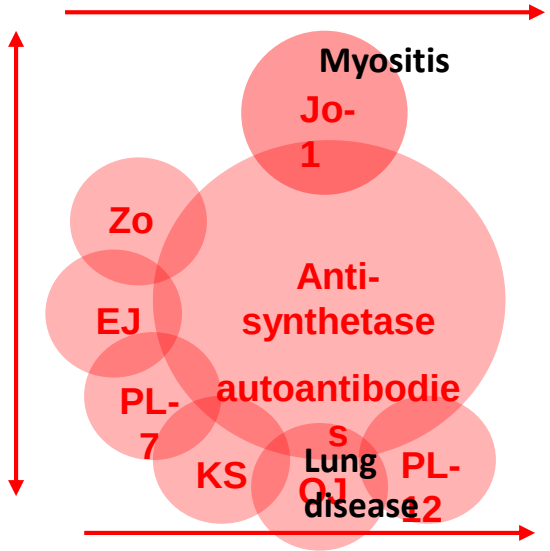


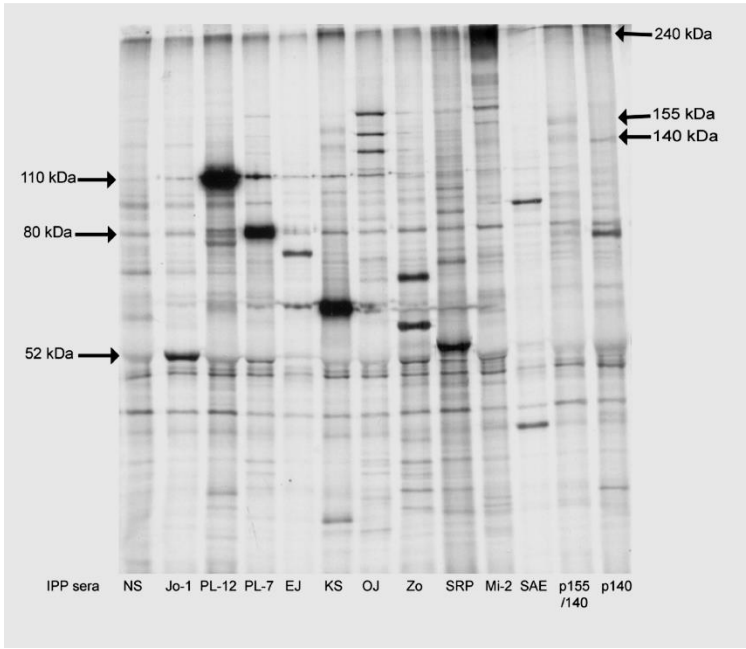






Autoantibody	tRNA synthetase target	Prevalence
Jo-1	Histidine	25-30%
EJ	Glycerine	<2%
PL-7	Threnyine	3-4%
KS	Asparigine	<2%
OJ	Isoleucine	<2%
PL-12	Alanine	3-4%
Zo	Phenylalanine	<2%



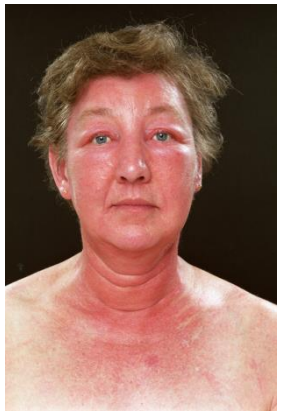


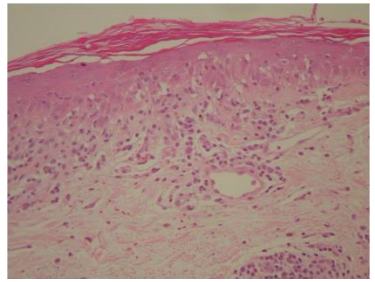
Learning points from Case A

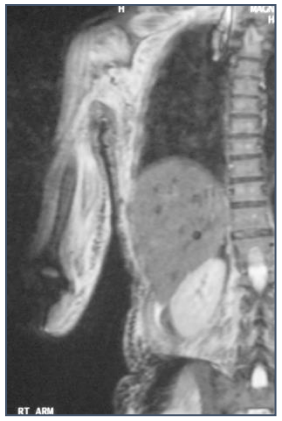
- Interstitial lung disease may be the predominant or even only manifestation of myositis (anti-synthetase syndrome)
- Autoantibodies can be missed as they do not give a strong ANA on routine screening
- Multidisciplinary management is essential in myositis
- Case A would not have fulfilled older criteria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

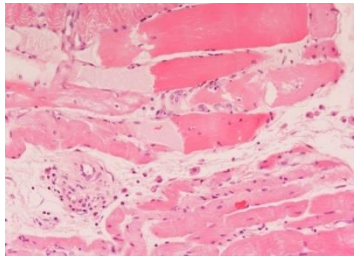
Case C female born 1949

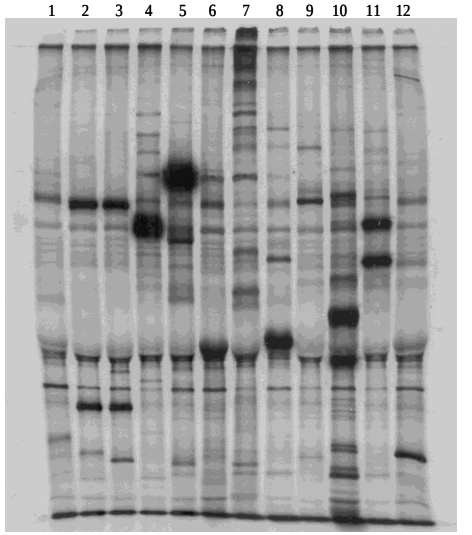
- 2002
  - Rash face
  - Biopsy lupus/DM
  - Rx Predisolone and HCQ
  - 6 months later weakness and dysphagia
  - CK 797
  - ANA anti-SAE



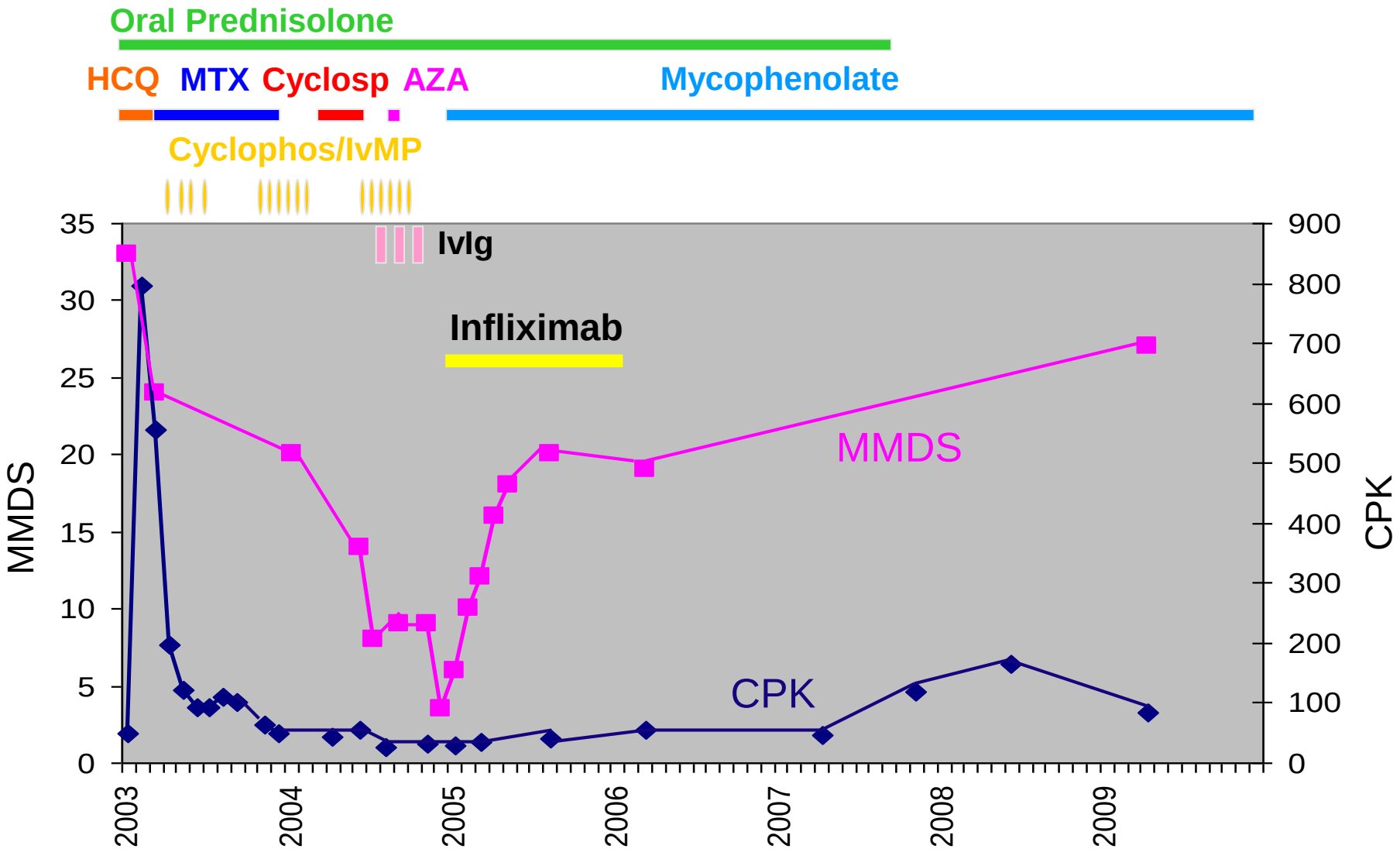








Case C 2003-2009



A new autoantibody marker in adult dermatomyositis

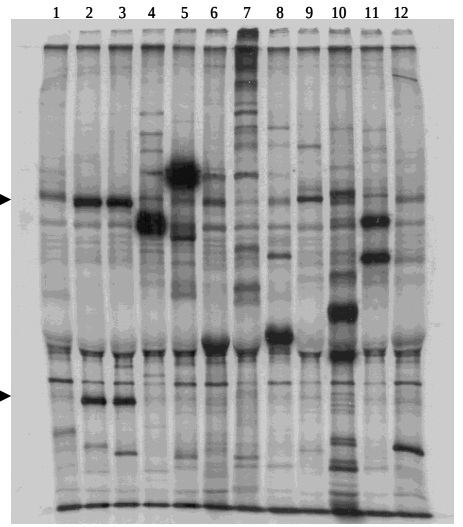
ARTHRITIS & RHEUMATISM
Vol. 56, No. ●, Month 2007, pp 000–000
DOI 10.1002/art.●

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 Slinn,[3] and Neil McHugh[2]

Q9UBT2	ULE1B _Human	Ubiquitin like 1 activating enzyme E1B SUMO1 (activating enzyme subunit 2)	71.179

Q9UBE0	ULE1A _Human	Ubiquitin like 1 activating enzyme E1A SUMO1 (activating enzyme subunit 1)	38.425



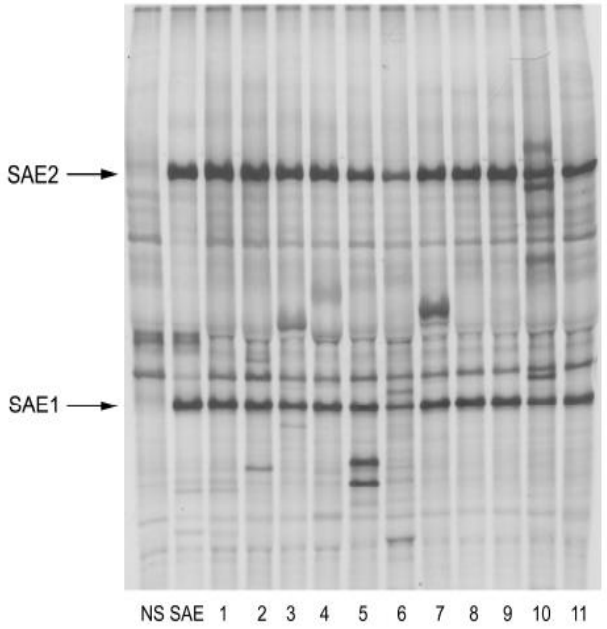
Learning points from Case C

- Example of a case of clinically amyopathic dermatamyositis (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

Ann Rheum Dis published online 17 Oct 2008;
doi:10.1136/ard.2008.097162



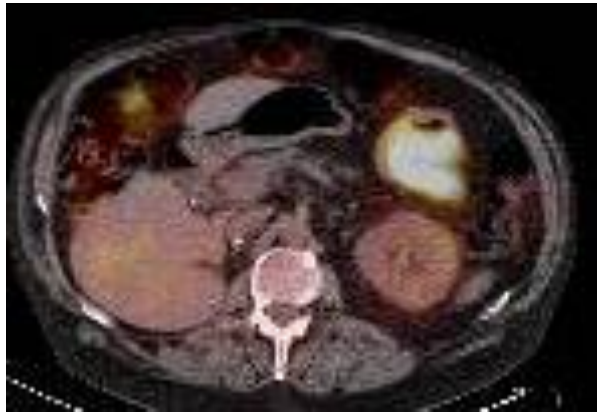
- 11 / 266 (4.9%) were positive for anti-SAE
- Found exclusively in 8.4 % of adult DM patients
- Specific clinical features
- Strong association with HLA DRB1*04/ DQB1*03

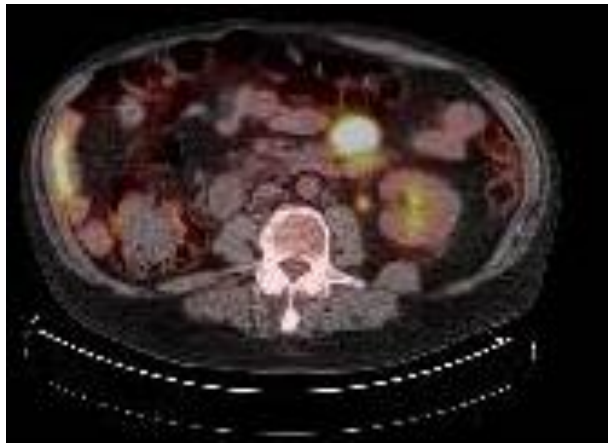
Case D male born 1953

- **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





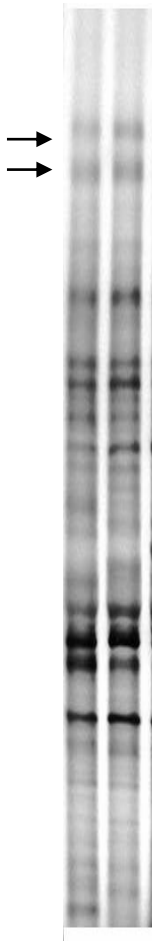
Cancer associated myositis

- **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

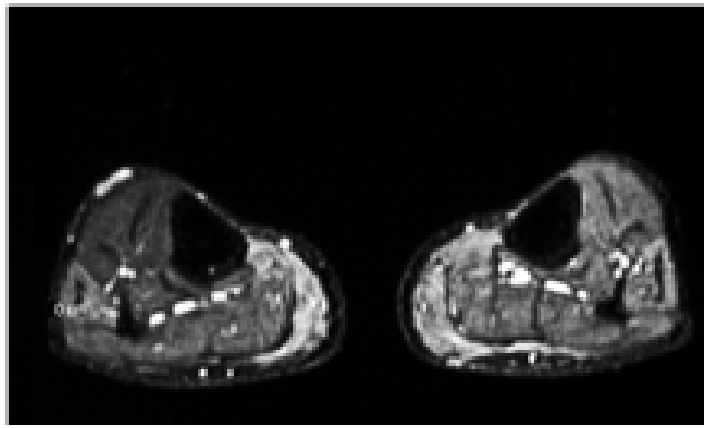
Targoff IN *et al.* Arthritis Rheum 2006;54:3682-3689.
Kaji K *et al*. Rheumatology 2007;46:25-28.
Gunawardena H *et al*. Ann Rheum Dis 2007, 66:S68.
Chinoy H *et al*. Ann Rheum Dis 2007;66:1345-1349.
Fujikawa K *et* al. Scan J Rheumatol 2009;38:263–267.
Trallero-Aragua´s E *at al*. Medicine (Baltimore) 2010;89:47–52.
Fujimoto M *et al*. Arthritis Rheum 2012;64:513-522

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

Clinical Feature	TIF1 Negative	TIF1 Positive	p value
Gottrons	29.7%	79.3%	<0.0001
Heliotrope Rash	29.3%	77.3%	<0.0001
ILD	31.2%	16.0%	=0.0038
Cancer (ever)	8.0%	32.2%	<0.0001
CAM	2.3%	20.5%	<0.0001
CADM	0.8%	5.2%	=0.0028

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



Acknowledgements

Bath

Dr Zoe Betteridge

Dr S Tansley

R Palmer

A Thomas

C Cavill

J Dunphy

Dr H Gunawardena

UK

Prof R Cooper

Dr H Chinoy

Prof L Wedderburn

Dr P Gordon

P New

Europe

Prof J Vencovsky

Dr H Mann

Dr L Pleštilová

Prof I Lundberg

UK / EuMyoNet investigators

JDRR investigators

Patients & families

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The Cathal Hayes Foundation