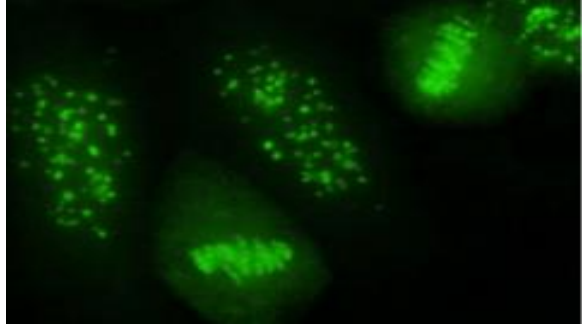
UNIVERSITY OF BATH



Secrets revealed by myositis autoantibodies

Neil McHugh, University of Bath and

Royal National Hospital for Rheumatic Diseases

Louisville September 2018

Royal National Hospital for Rheumatic Diseases NHS Foundation Trust

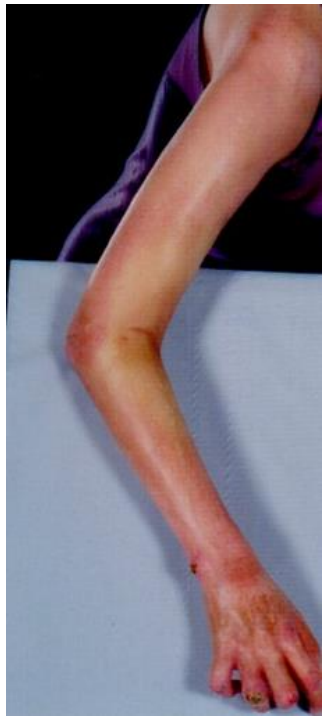
THE MYOSITIS ASSOCIATION

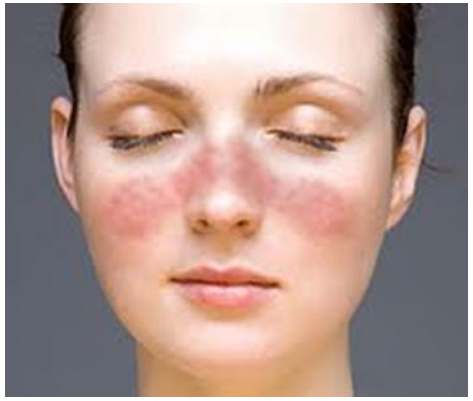
The origins of ‘rheumatic’ disease

Rheuma – Greek for flow; Rheumatism – to suffer from a ‘flux’

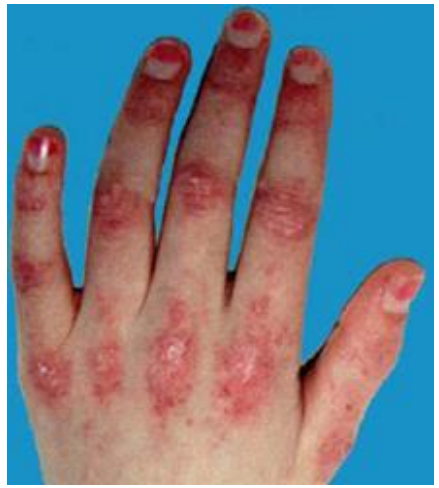
The ‘flux’ of ‘rheumatic’ disease

Scleroderma

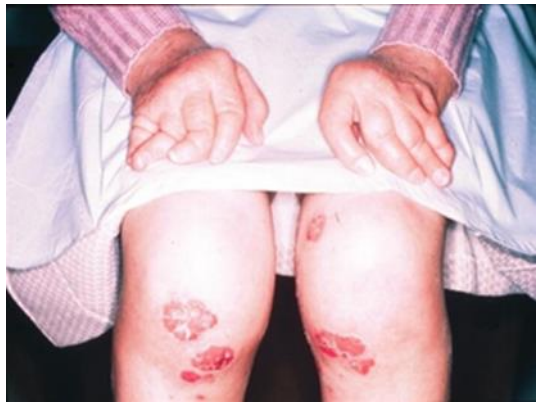




Systemic lupus erythematosus



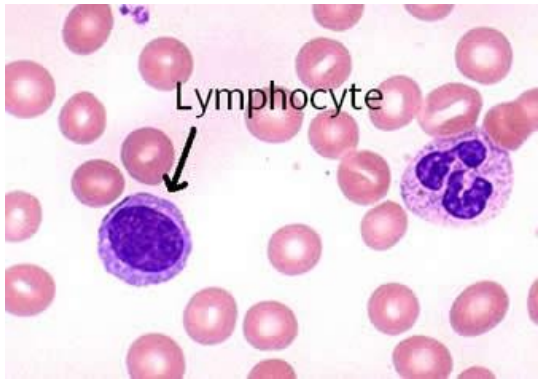
Dermatomyositis

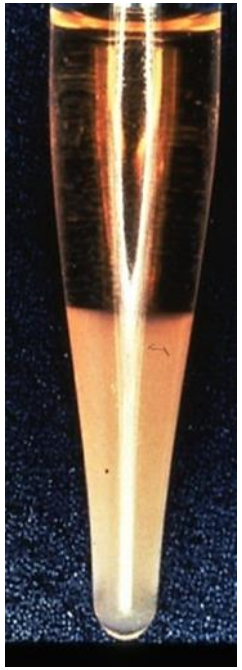


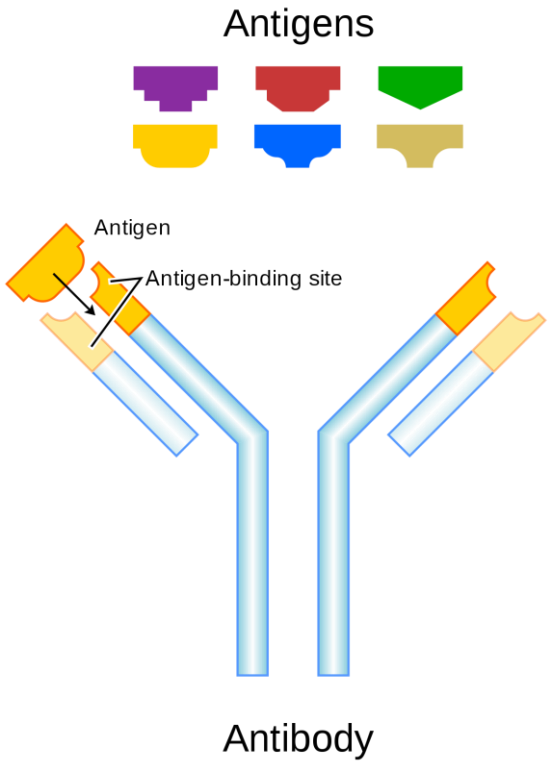
Psoriatic arthritis

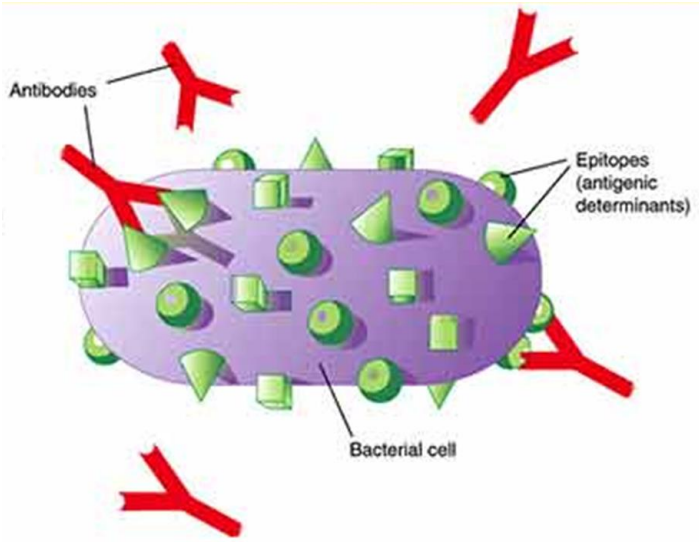
Antibodies are part of the ‘flux’ of the immune system

B lymphocytes make antibodies

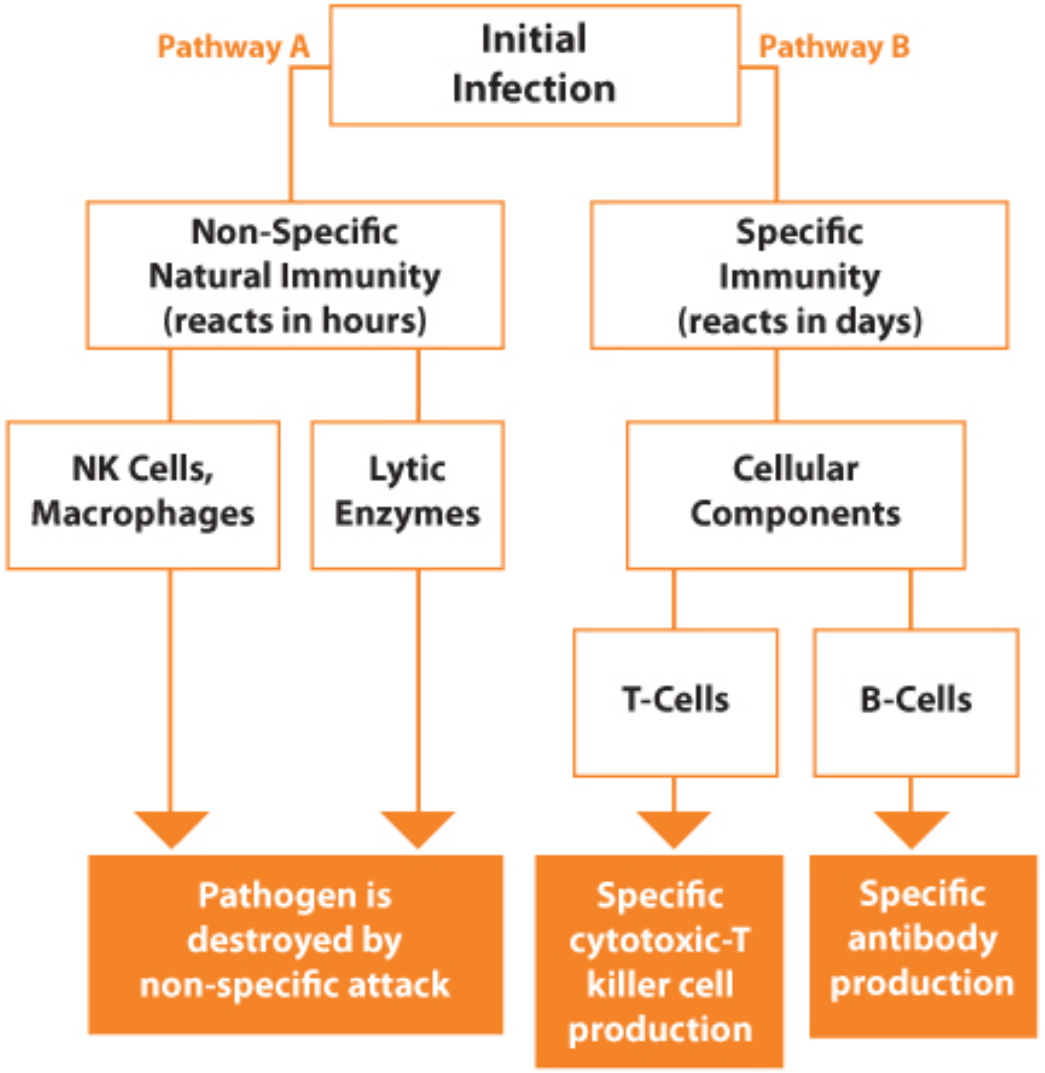




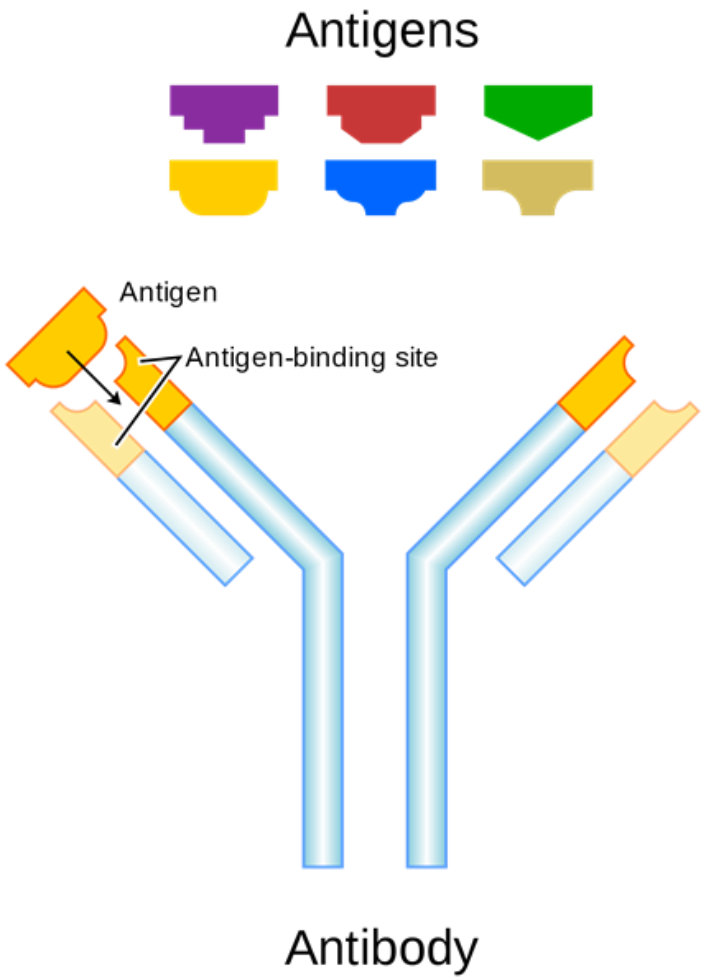




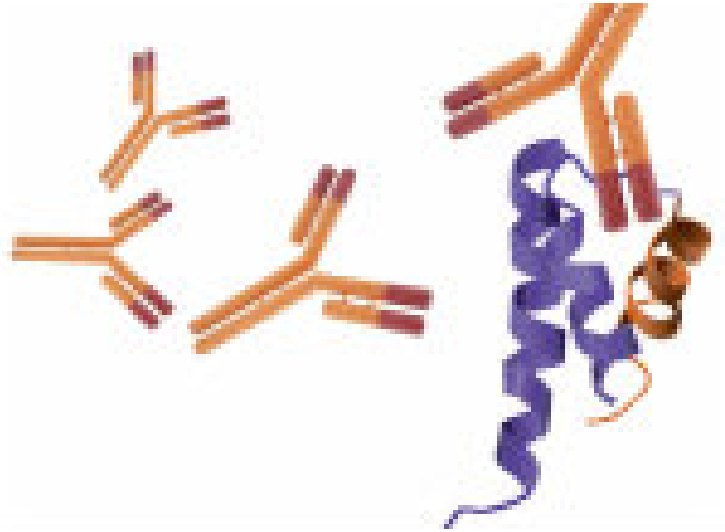
The immune system (innate and adaptive)



Antibodies and Autoantibodies



Immunoglobulin

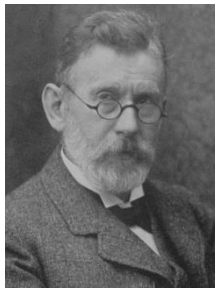


Autoantibody (anti-DNA)

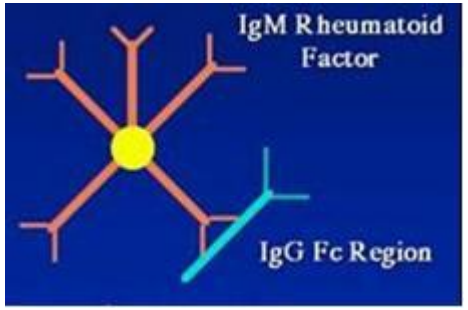
Autoantibodies are antibodies that recognise self-constituents (autoantigens) rather than foreign particles

Autoantibodies are the hallmark of autoimmunity

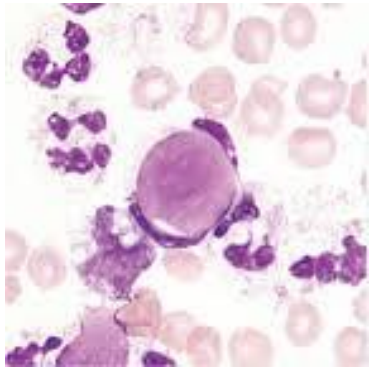
Autoimmunity



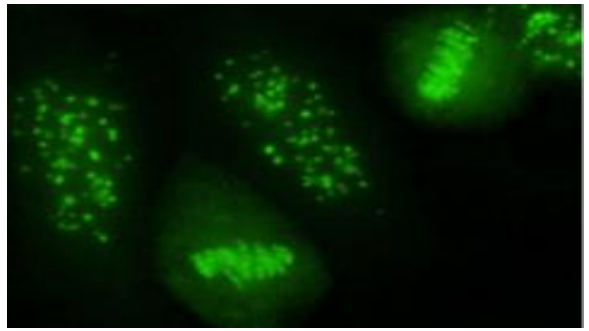
- 1903
  - **Paul Ehrlich** 'horror autotoxicus'
- 1943-1946
  - **Eric Waaler and Harry Rose** described Rheumatoid factor



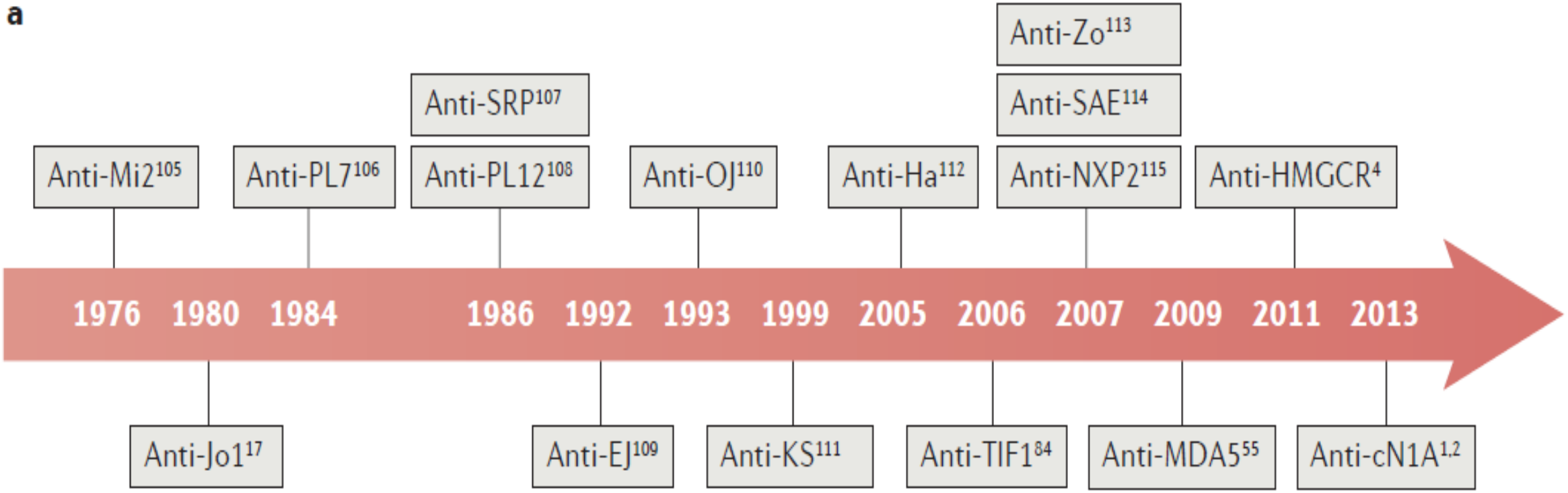
- 1948
  - **Hargreaves.** LE cell



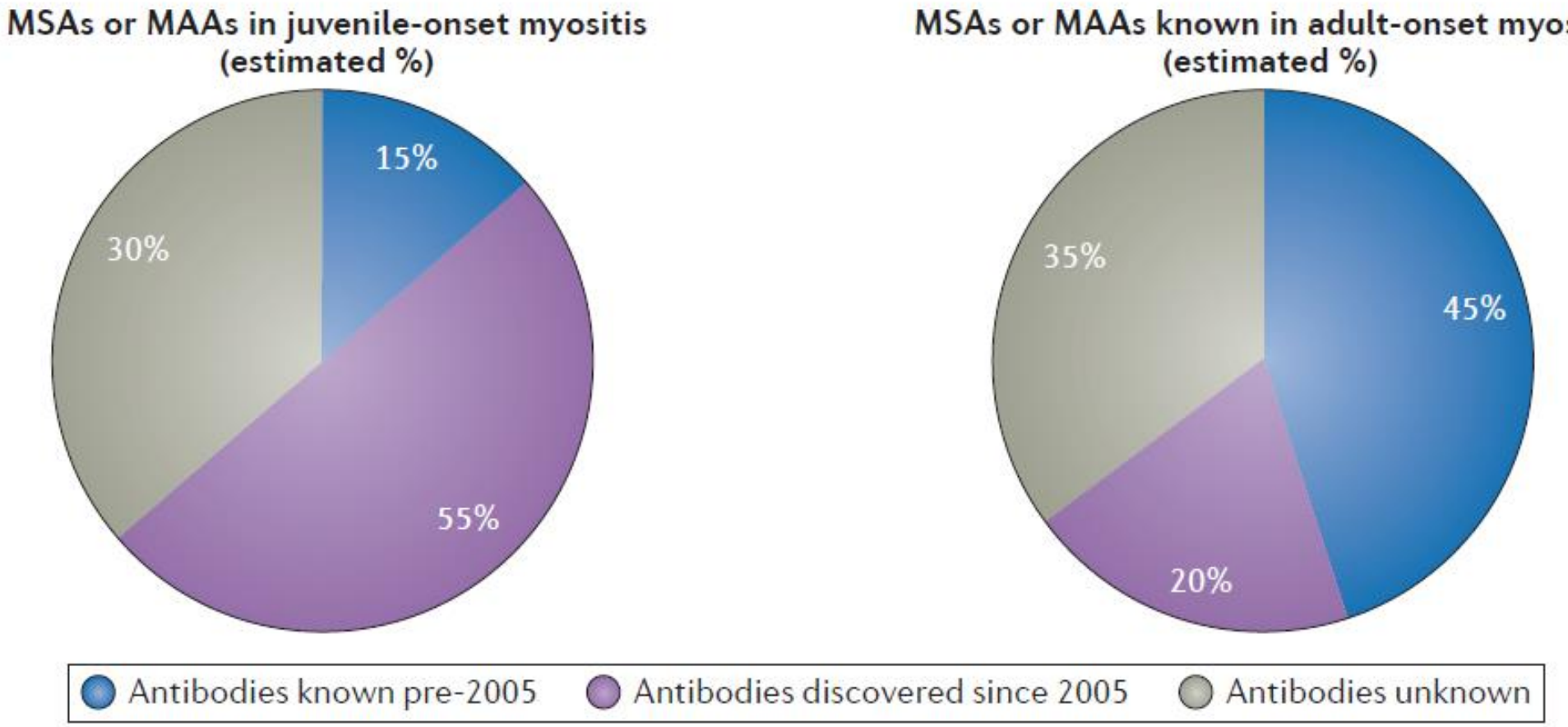
- !966
  - **Tan & Kunkel** described anti-Sm
- 1976
  - **Reichlin** described anti-Mi2
- 1980
  - **Moroi** et al described anti-centromere antibodies in scleroderma



Timeline of myositis specific autoantibody discovery



Since 2005 the majority of juvenile and adult myositis cases have an identifiable myositis autoantibody



So our journey begins…

- Wellington Rheumatology trainee 1982-1984
- RACP Grant Melbourne 1985
- Dorothy Eden Fellowship RNHRD, Bath 1985-1986
- Senior Registrar RNHRD 1987-1990

The Australian experience

FIAT LUX

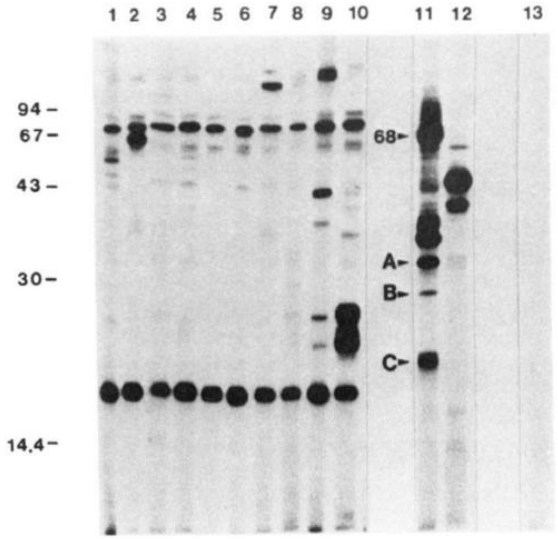
Walter+ Eliza Hall

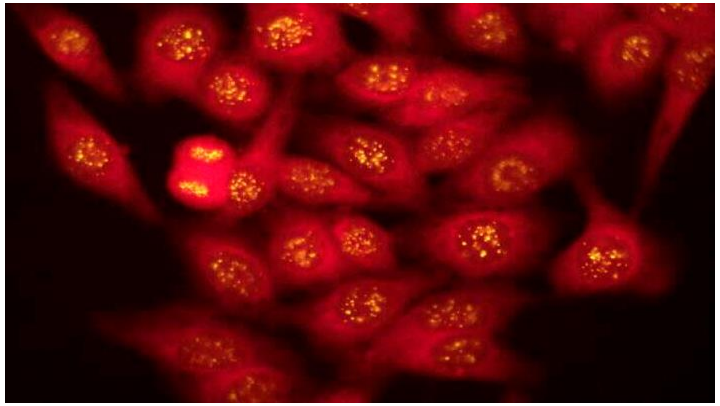
Institute of Medical Research

A HIGHLY CONSERVED 72,000 DALTON CENTROMERIC ANTIGEN REACTIVE WITH AUTOANTIBODIES FROM PATIENTS WITH PROGRESSIVE SYSTEMIC SCLEROSIS

L. JANE McNEILAGE,[1] SENGA WHITTINGHAM,[2] NEIL McHUGH,[3] AND ALFRED J. BARNETT[4]

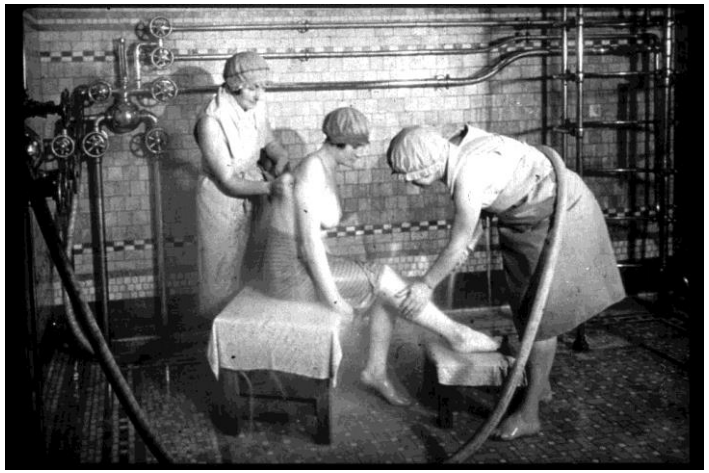
From The Clinical Research Unit of The Walter and Eliza Hall Institute of Medical Research and the Royal Melbourne Hospital, Post Office Royal Melbourne Hospital, Victoria 3050, Australia





Journal Immunology 137, 2541-2547 1985

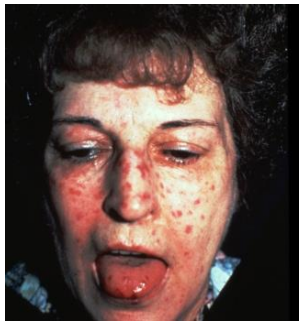
The golden age of treatment at ‘The Min’



Perkins tractors

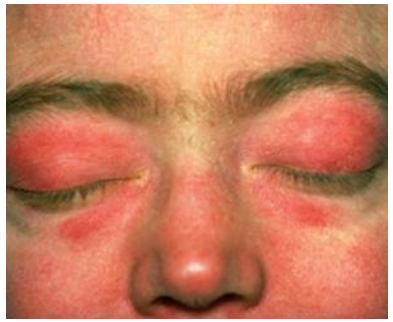
- Cure for gout, rheumatism, headaches and epilepsy
- Distributed in England by a Bath Physician with a thriving practice (also a superintendent of mental asylum)
- 1799 Dr Haygarth at the Min performed placebo study with fake wooden tractors on five patients
- *‘the wooden tractors were drawn over the skin such as to touch it in the lightest manner… distinctly proving to what a surprising degree mere fancy deceives the patient’*

The spectrum of autoimmune connective tissue disease



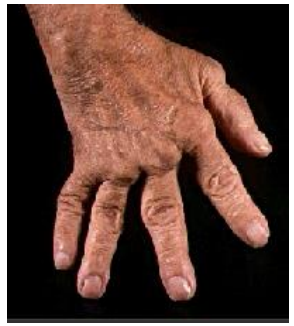
Scleroderma

Nucleolar RNP



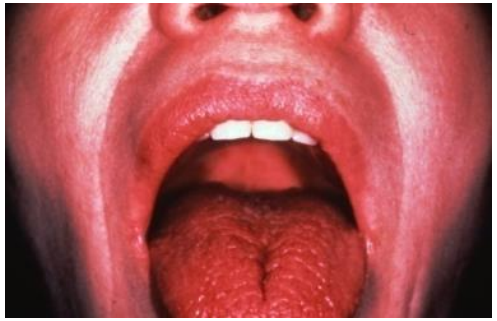
Dermatomyositis

Transcription factors
RNA synthetase



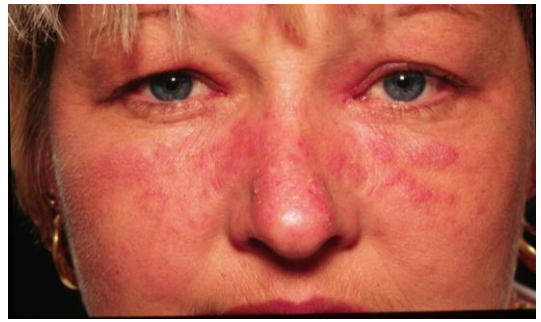
Rheumatoid arthritis

ACPA



Sjogren's

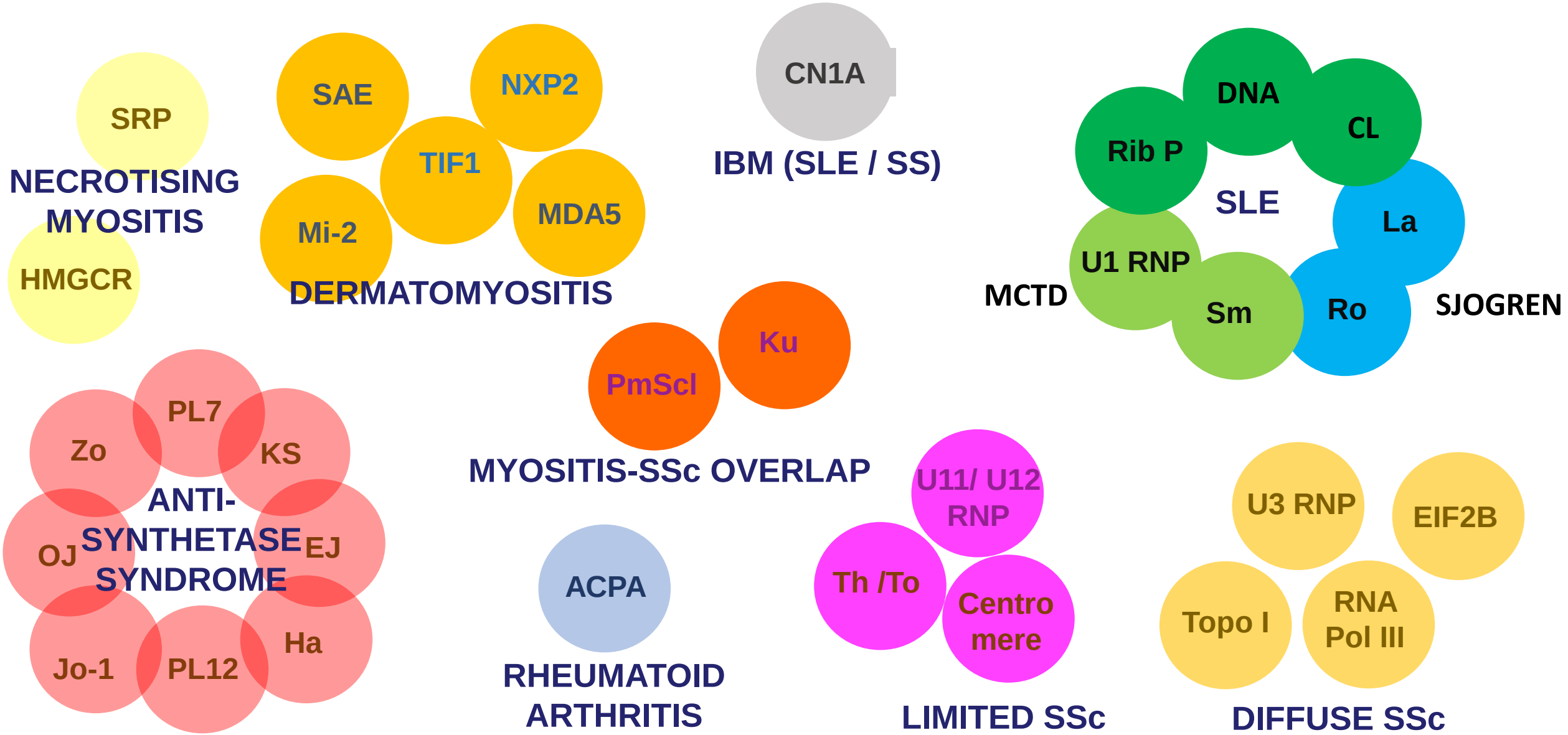
Ro/La (SS-A/SS-B)



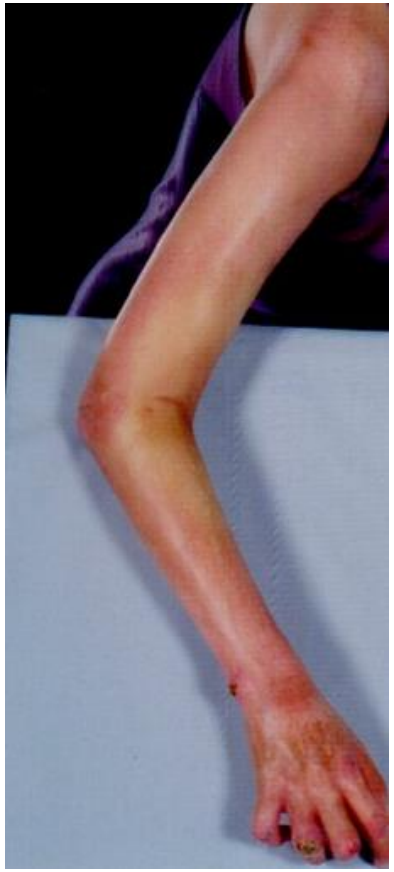
SLE

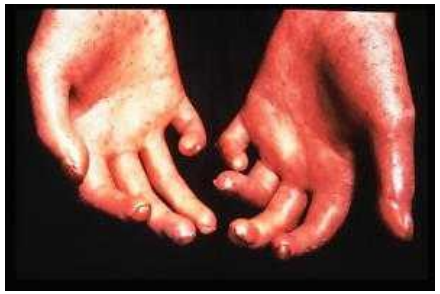
snRNPS
Nucleosome

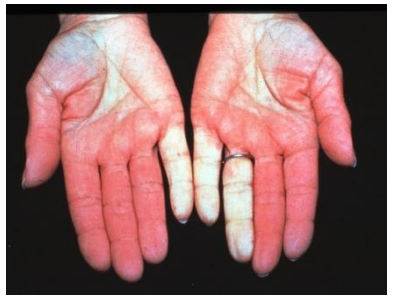
Autoantibodies in CTD

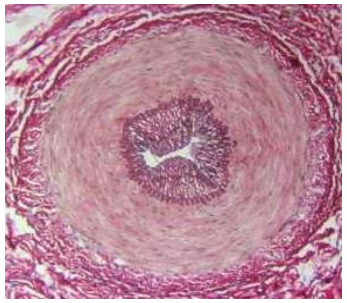


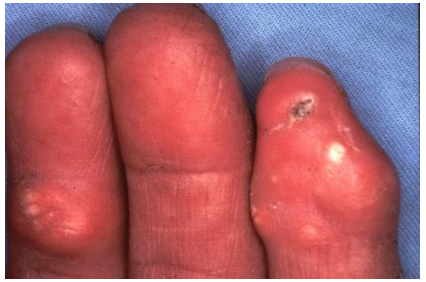
Scleroderma (systemic sclerosis)

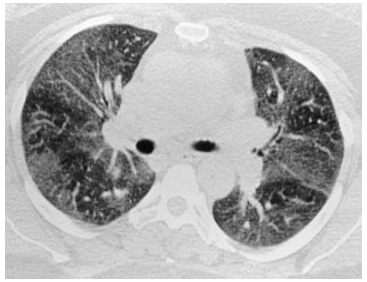






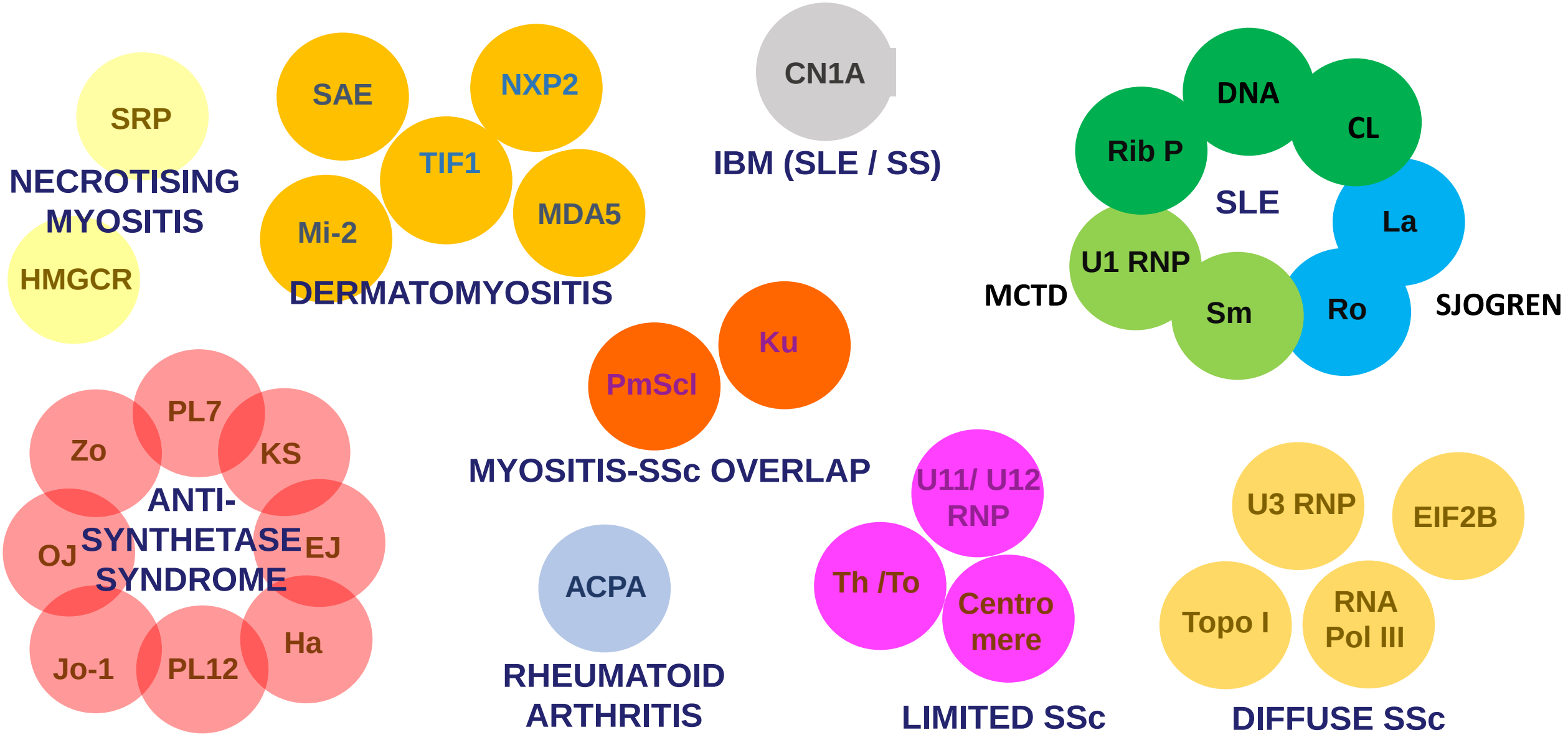




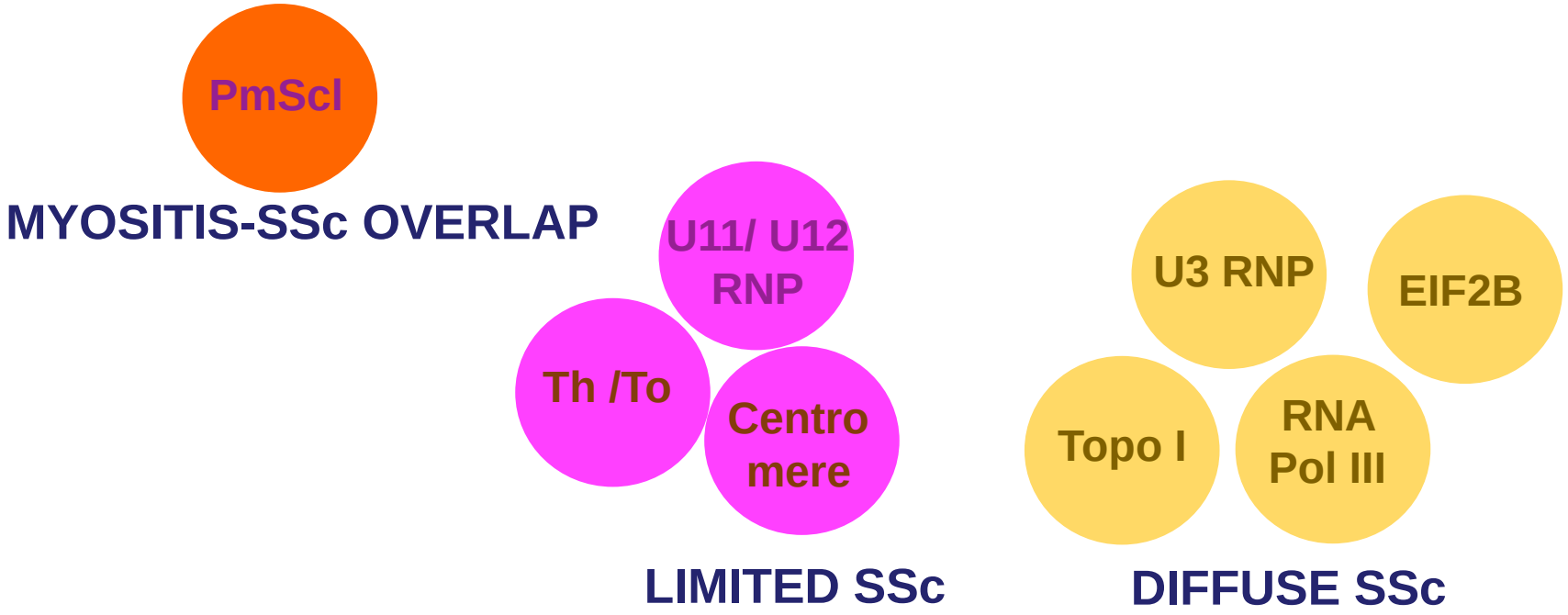


- Abnormal accumulation of collagen and other matrix proteins in affected tissue
- Mainly affects
  - Skin
  - Blood vessels
  - Lungs, Kidneys, Gut
- Presence of disease specific autoantibodies

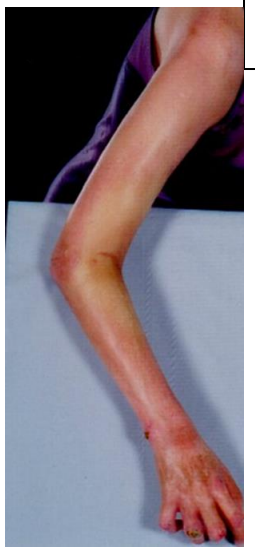
Autoantibodies in CTD



Autoantibodies in scleroderma



Serological subsets in scleroderma



Diffuse skin disease

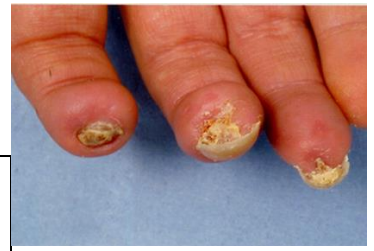
Lung

EIF2b

Topo-I

Centromere

Limited skin disease



RNAP

PHT

Kidney

Lung

U3RNP

Th RNP

U1RNP

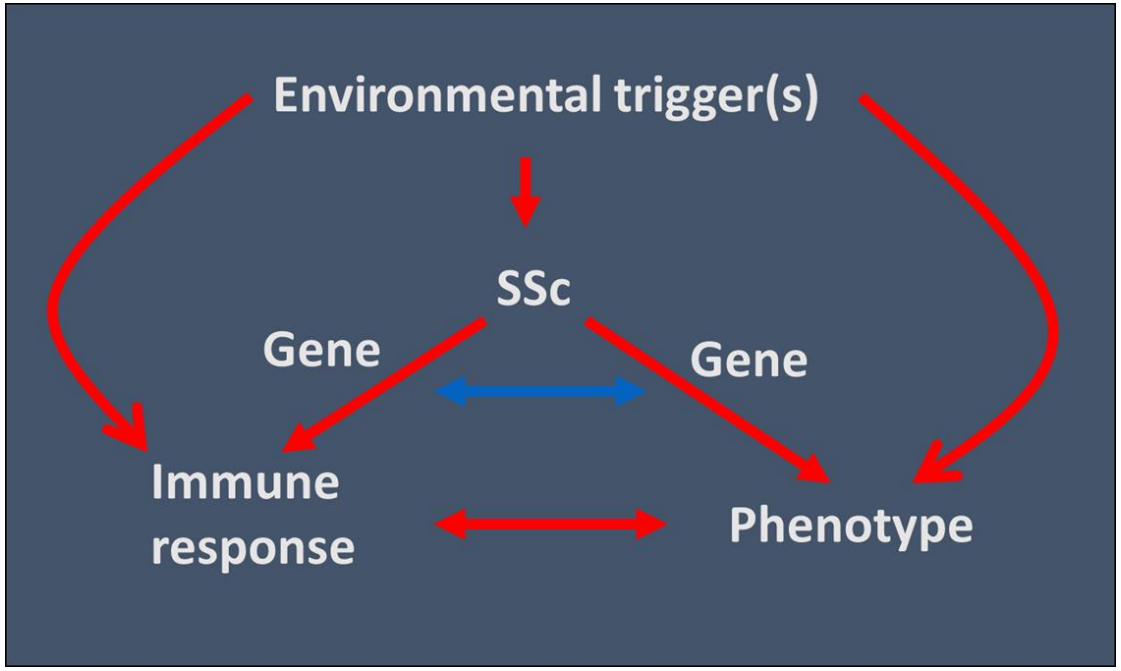
Pm-Scl

Overlap features

SEGREGATION OF AUTOANTIBODIES WITH DISEASE IN MONOZYGOTIC TWIN PAIRS DISCORDANT FOR SYSTEMIC SCLEROSIS

Three Further Cases

NEIL J. McHUGH, GEORGINA R. HARVEY, JEAN WHYTE, and J. KEVIN DORSEY

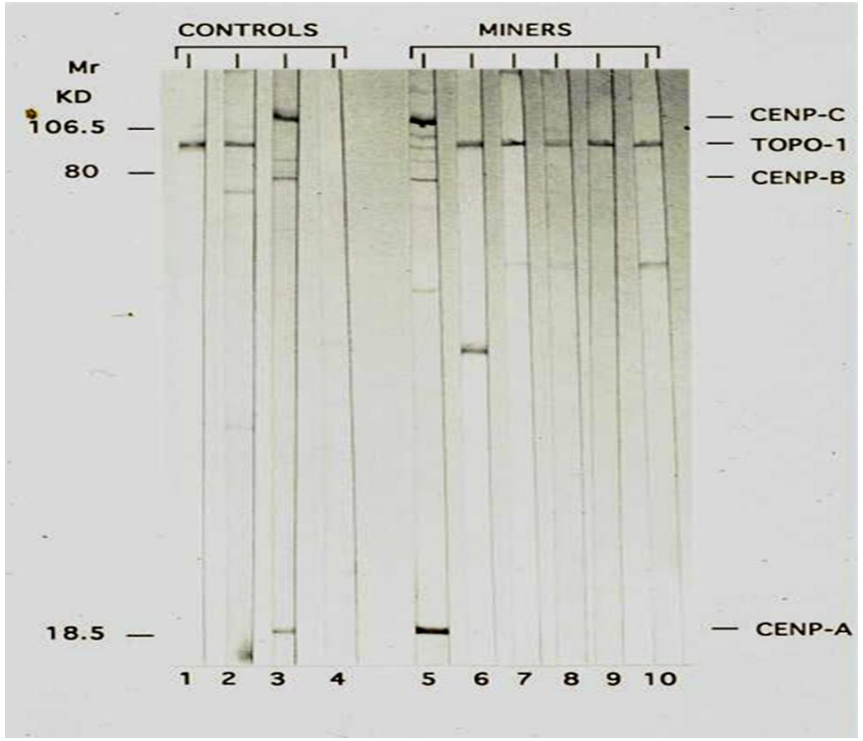


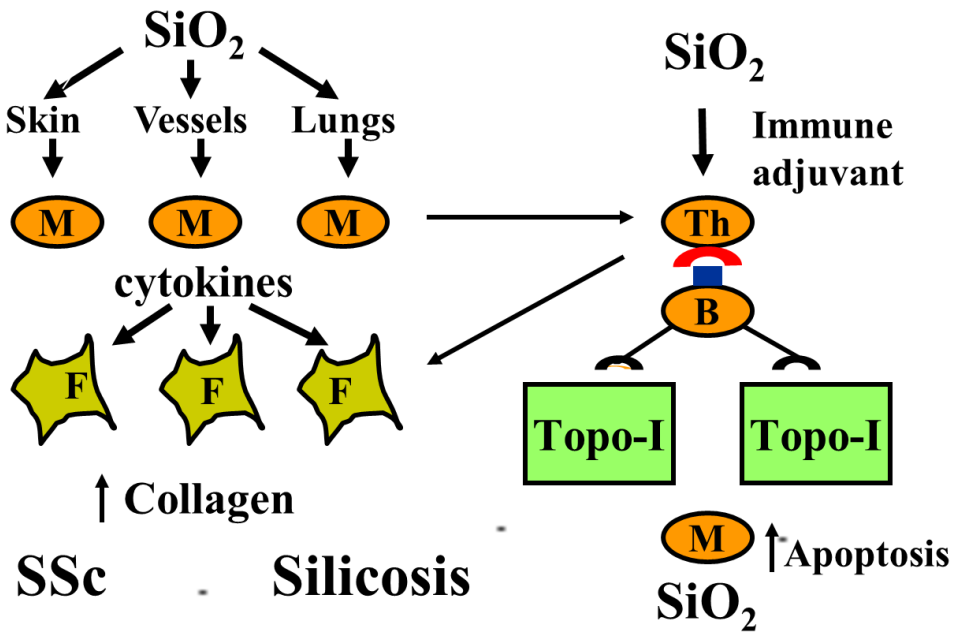
Arthritis Rheum. 1995

ANTI–TOPOISOMERASE I ANTIBODIES IN SILICA-ASSOCIATED SYSTEMIC SCLEROSIS

A Model for Autoimmunity

NEIL JOHN McHUGH, JEAN WHYTE, GEORGINA HARVEY, and UWE F. HAUSTEIN





Arthritis Rheum. 1994

.....the journey continues

The American dream

Proc. Natl. Acad. Sci. USA
Vol. 76, No. 11 pp. 5495–5499, November 1979
Biochemistry

Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus

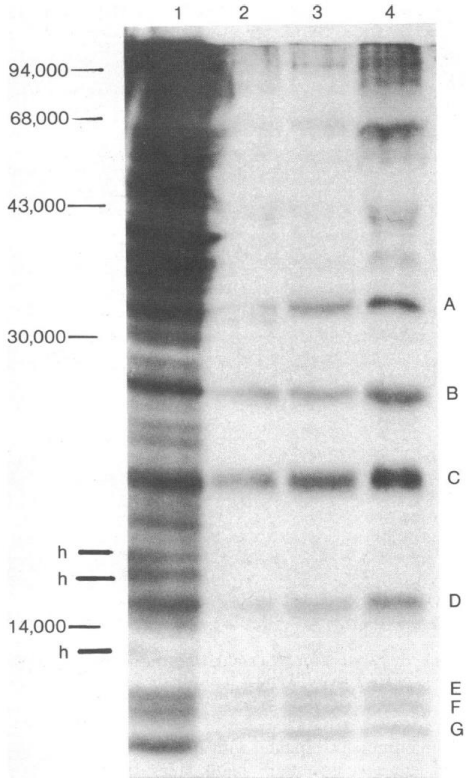
(nuclear ribonucleoprotein/rheumatic disease/RNA processing)

MICHAEL RUSH LERNER AND JOAN ARGETSINGER STEITZ

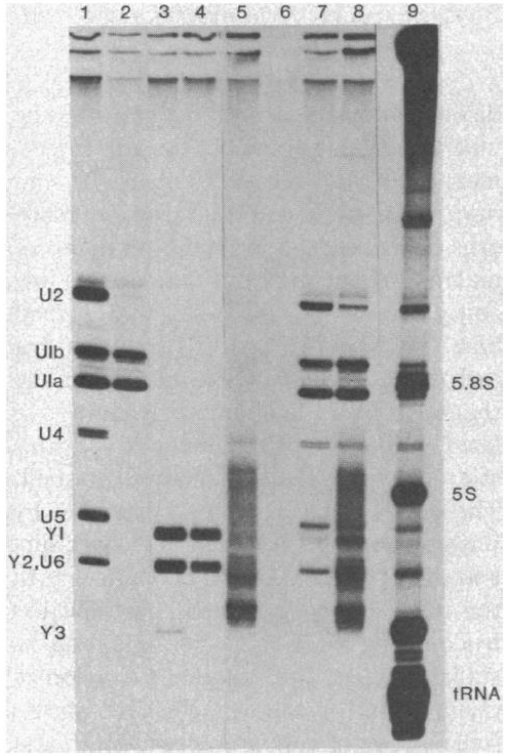
Two Novel Classes of Small Ribonucleoproteins Detected by Antibodies Associated with Lupus Erythematosus

Abstract. *The RNP and Sm antigens recognized by lupus erythematosus antibodies are located on discrete particles containing single small nuclear RNA's complexed with proteins. The antigens Ro and La are also on ribonucleoproteins. The small RNA's in ribonucleoproteins with Ro are discrete, like those associated with RNP and Sm; in contrast, ribonucleoproteins with La contain a striking highly banded spectrum of small RNA's from uninfected cells as well as virus-associated RNA from adenovirus-infected cells.*

Lerner, Boyle, Hardin, Steitz. Science 1981

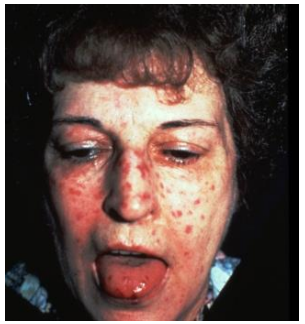


Protein autoantigens Isolated by immunoprecipitation



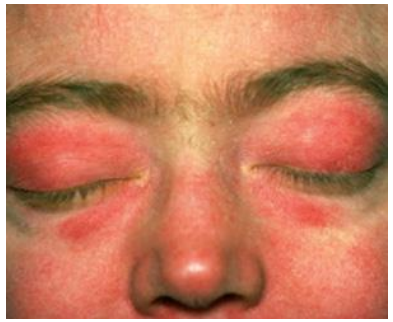
RNA species isolated by immunoprecipitation

The spectrum of autoimmune connective tissue disease



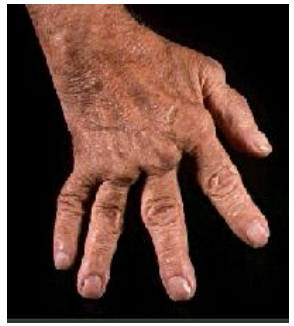
Scleroderma

Nucleolar RNP



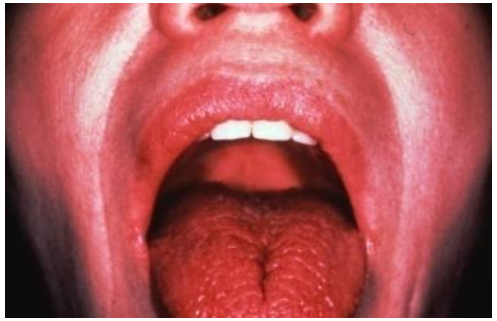
Dermatomyositis

Transcription factors
RNA synthetase



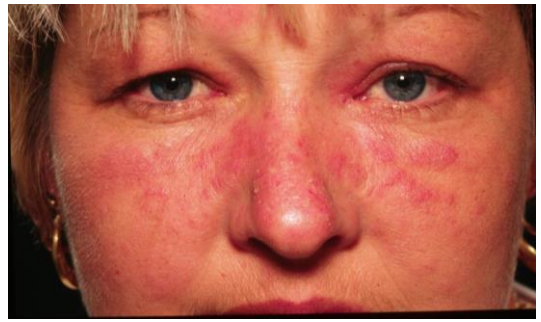
Rheumatoid arthritis

ACPA



Sjogren's

Ro/La (SS-A/SS-B)



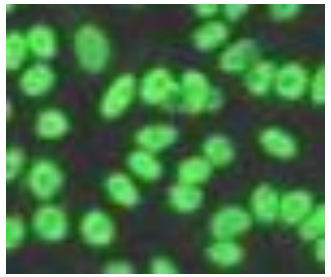
SLE

snRNPS
Nucleosome

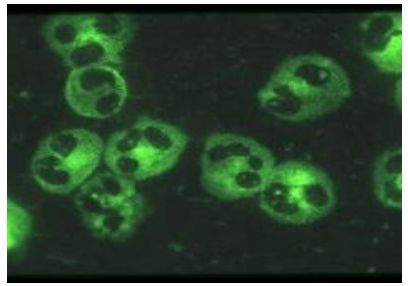
Methods for detecting autoantibodies

Autoantibody Screening by Indirect Immunofluorescence

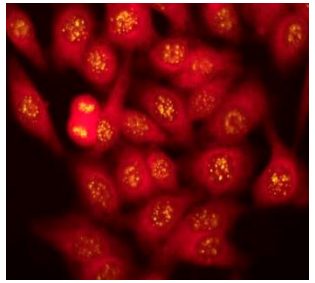
Hep-2



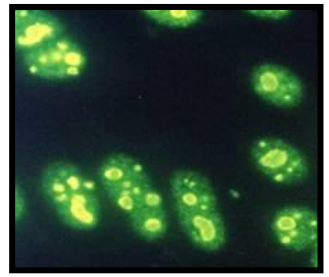
Human neutrophil



Hep-2

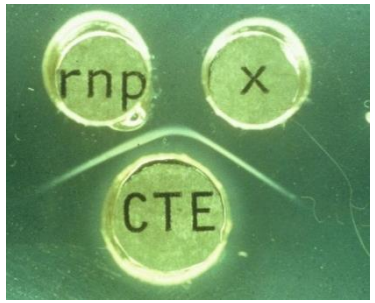


Hep-2



Autoantibody identification by second technique

Immunodiffusion

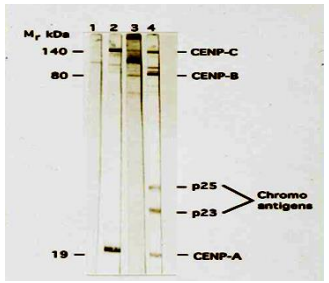


ENA
anti-RNP

ELISA

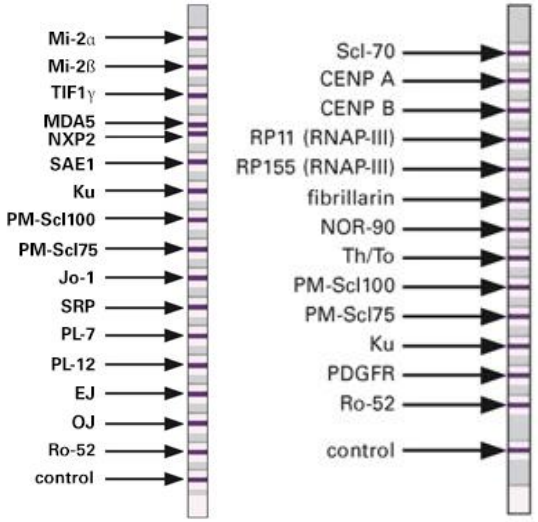
anti-PR3

Western blot

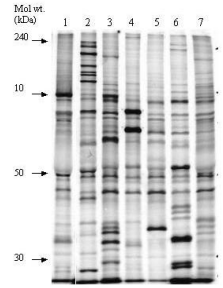


anti-centromere

Lineblot



Immunoprecipitation

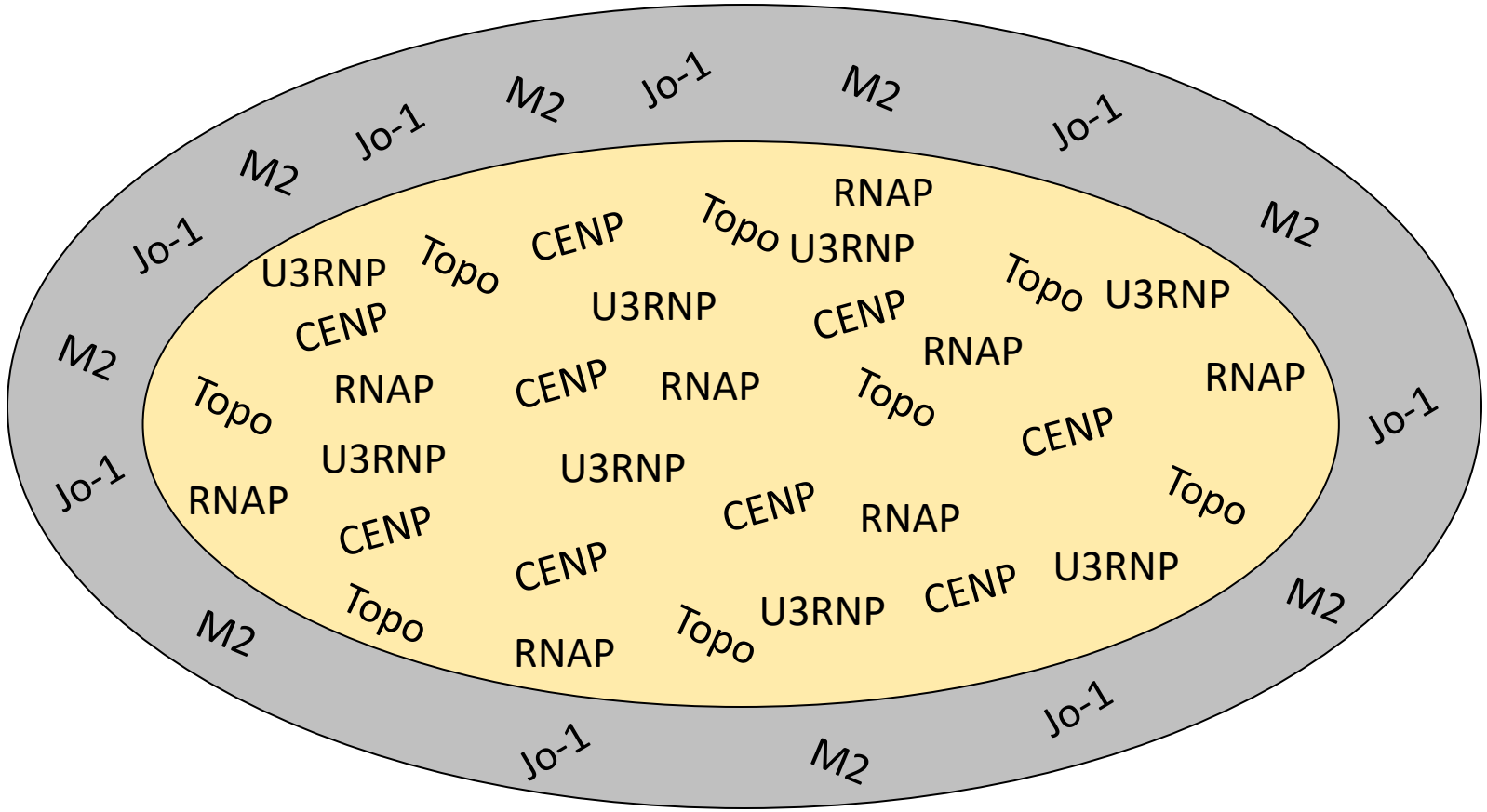


Anti-fibrillarin
U3RNP

Indirect Immunofluorescence

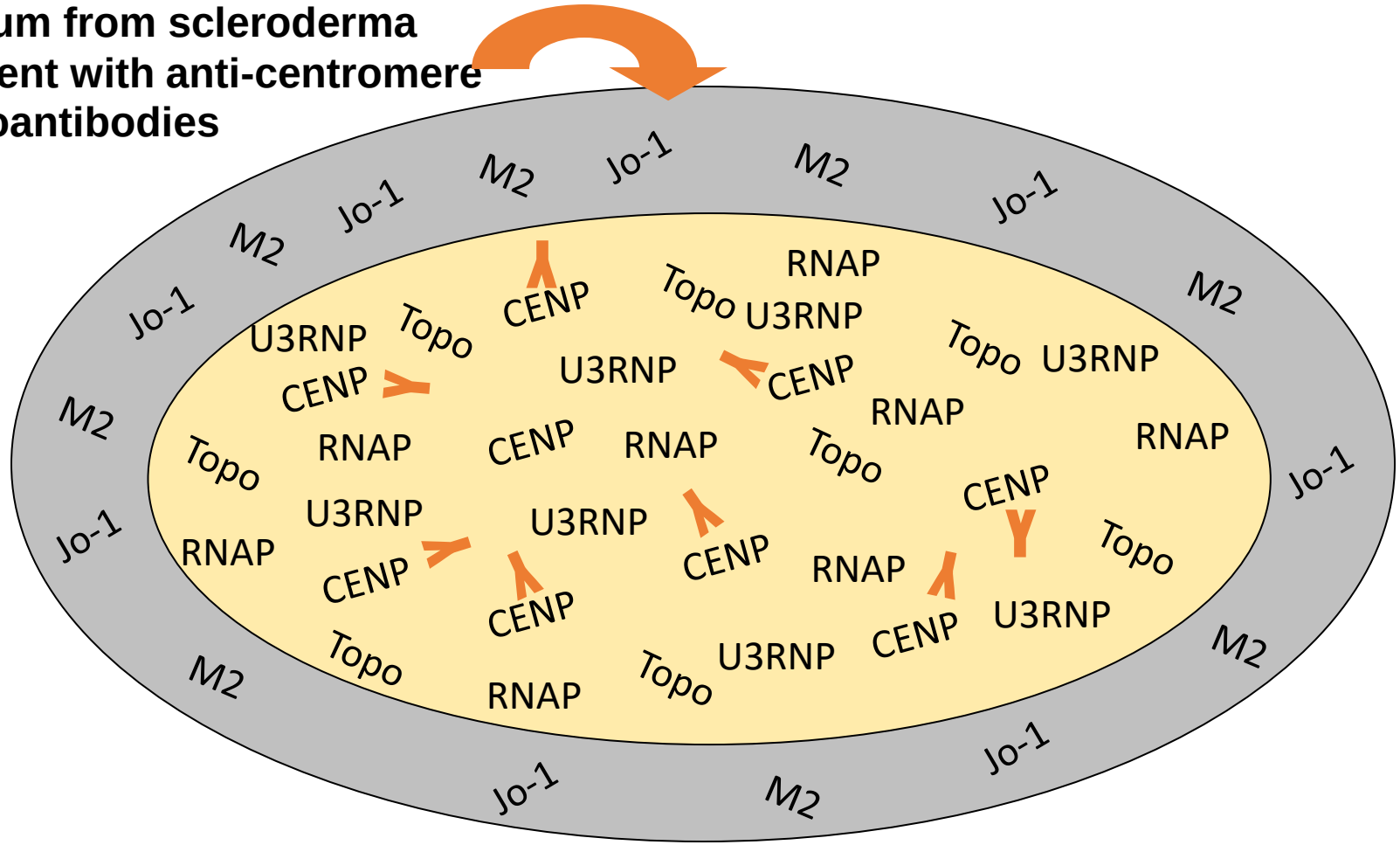
- **Antigen source -** **tissue section (mouse LKS, monkey oesophagus) whole cell (HEp-2, neutrophil, *crithidia luciliae*)**
- **Autoantibody from patient serum - Apply autoantibody that if present will bind to the antigen source**
- **Secondary antibody - anti-human IgG FITC**
- **Visualization - green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

Indirect immunofluorescence test I

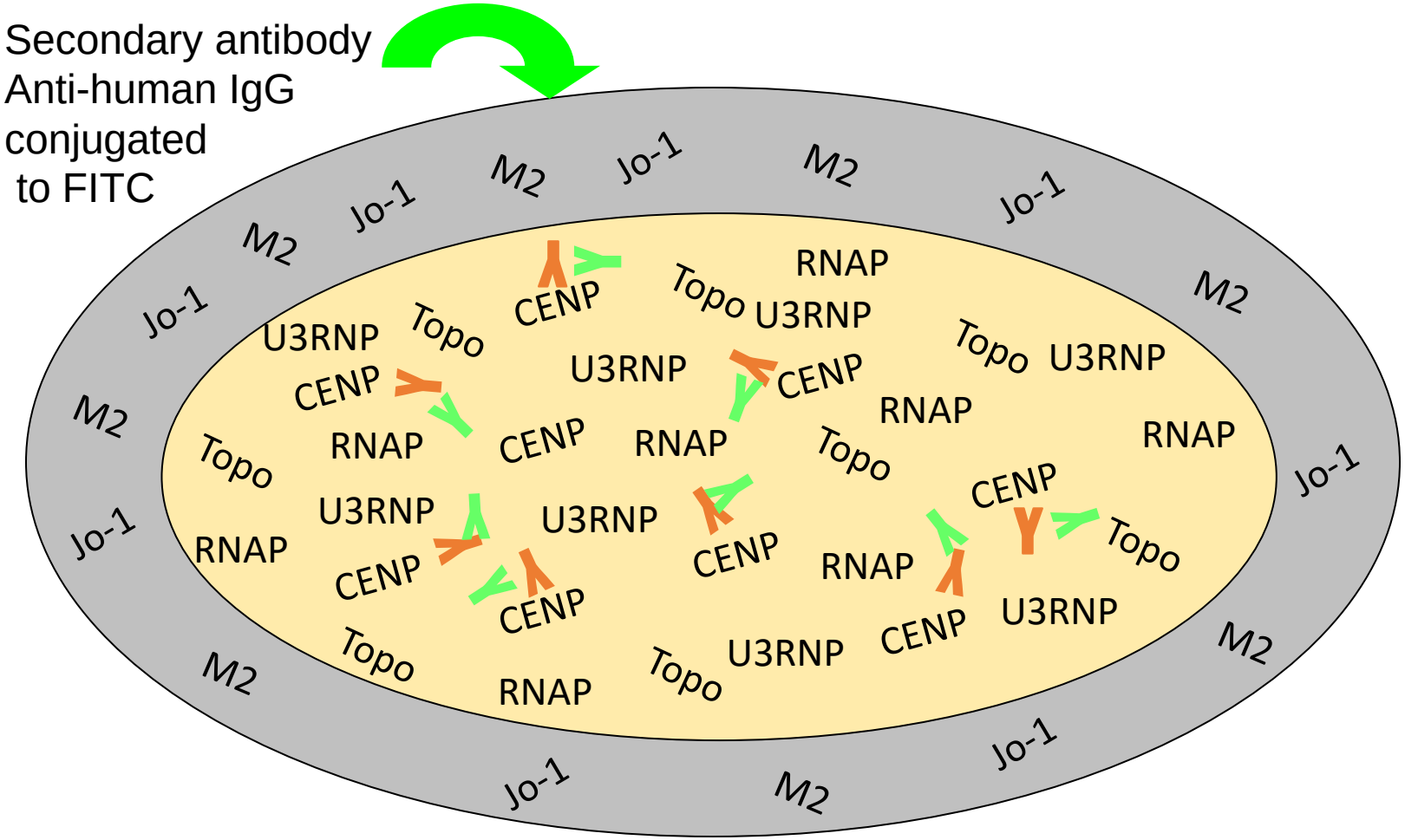


Indirect immunofluorescence test II

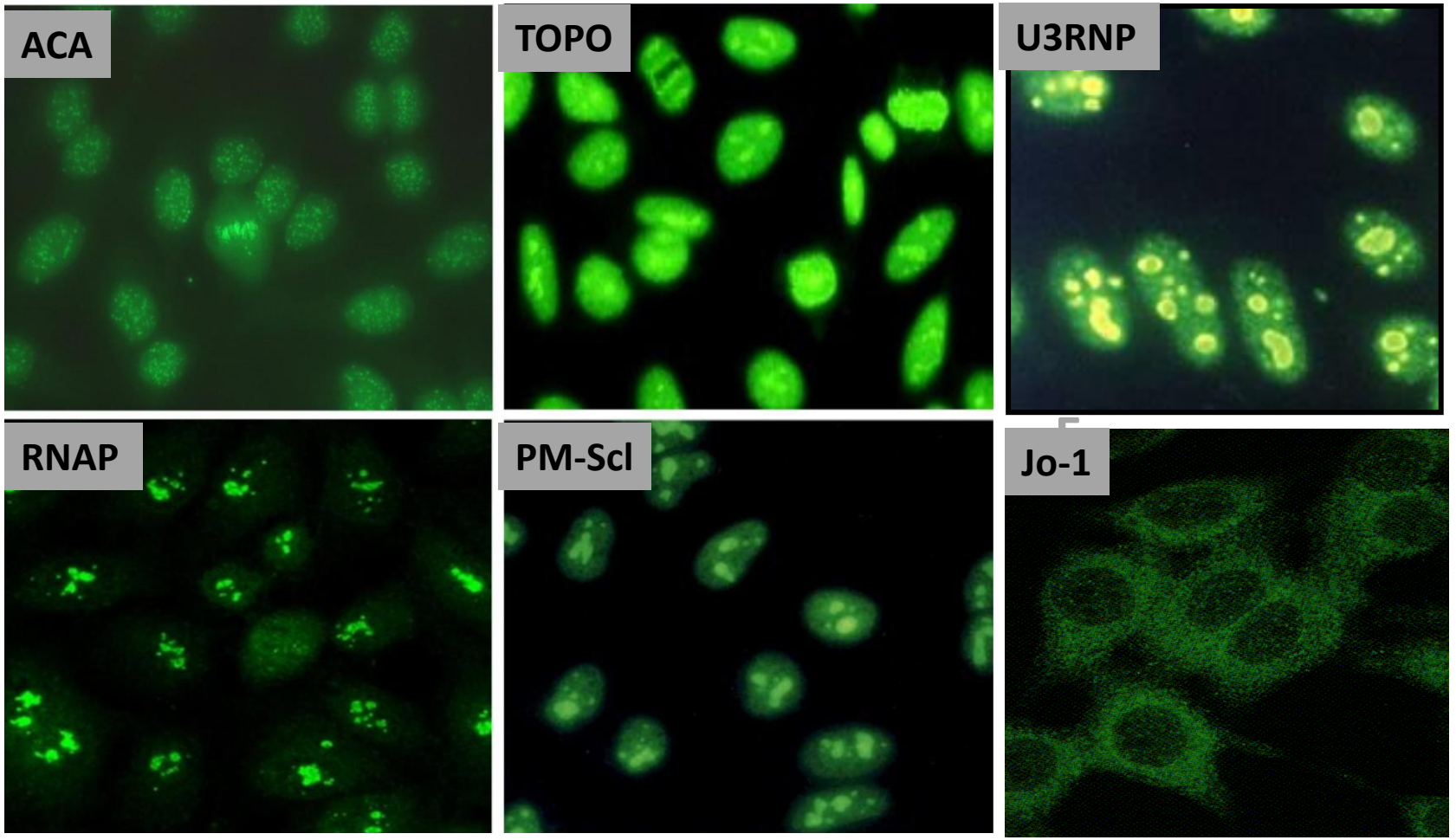
Serum from scleroderma patient with anti-centromere autoantibodies



Indirect Immunofluorescence test III

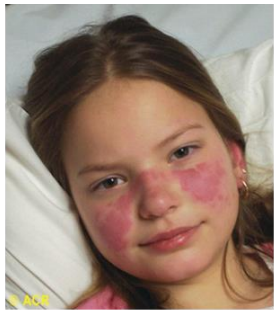


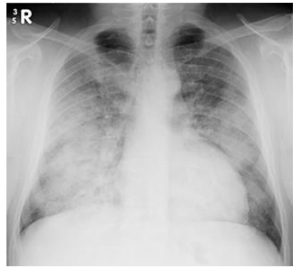
Indirect immunofluorescence

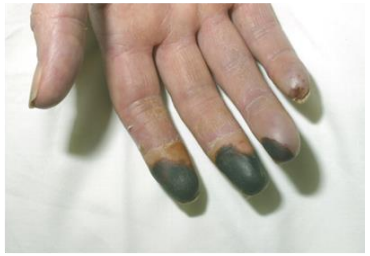


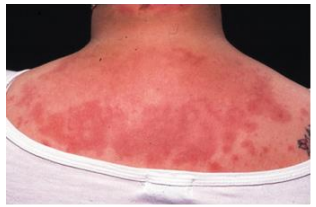
- If test positive the patient will be reported as having an antinuclear antibody (ANA)
- Sometimes the pattern will reveal the type of ANA (specificity) but usually another method will be necessary for exact identity

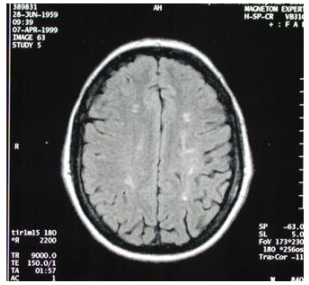
Systemic Lupus Erythematosus

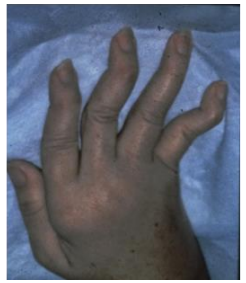


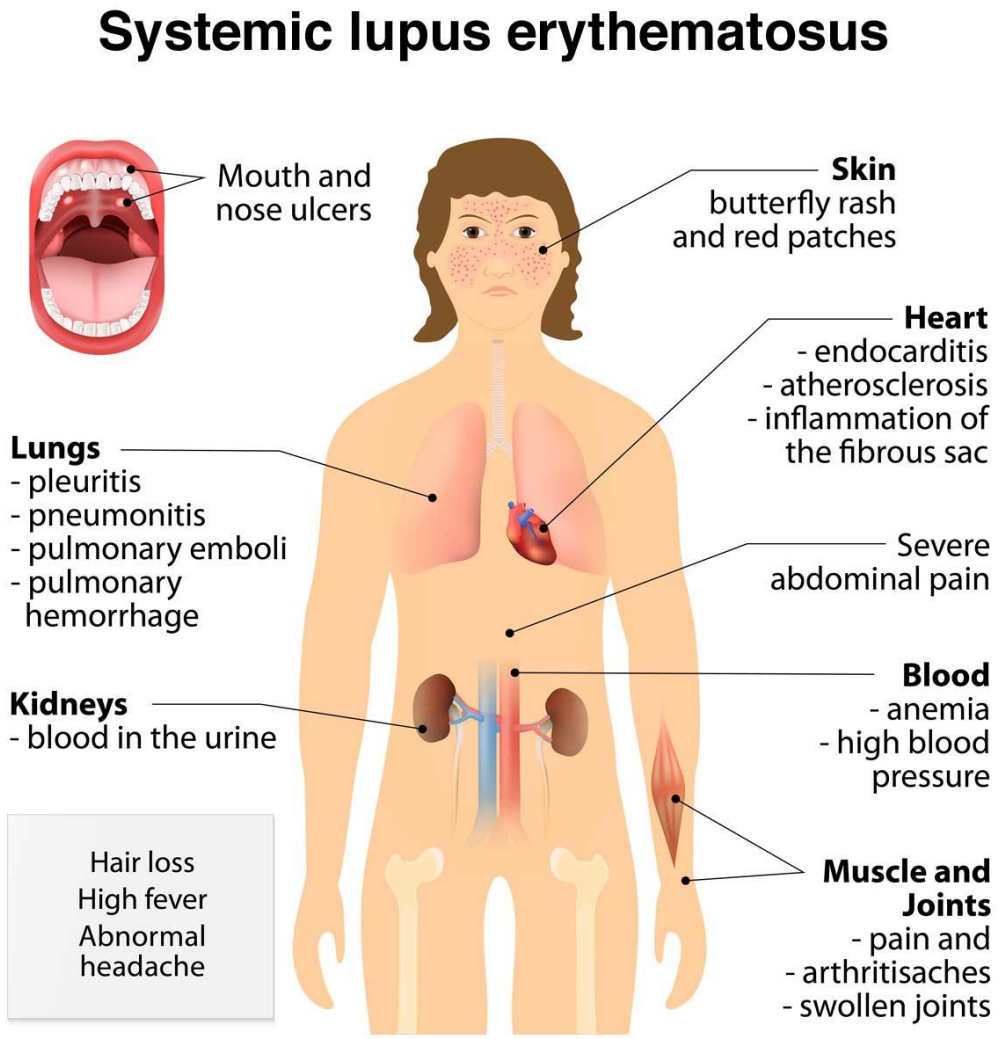




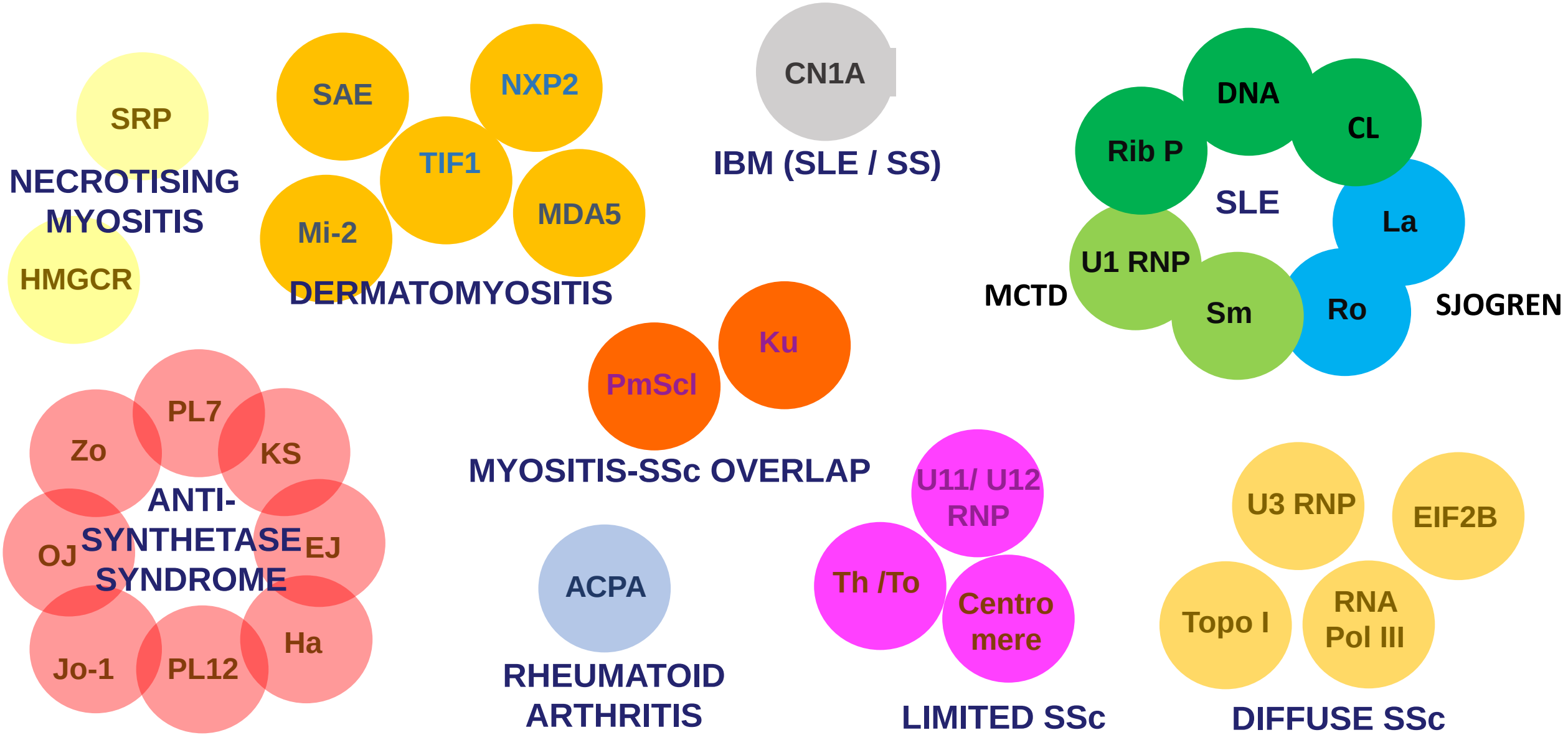




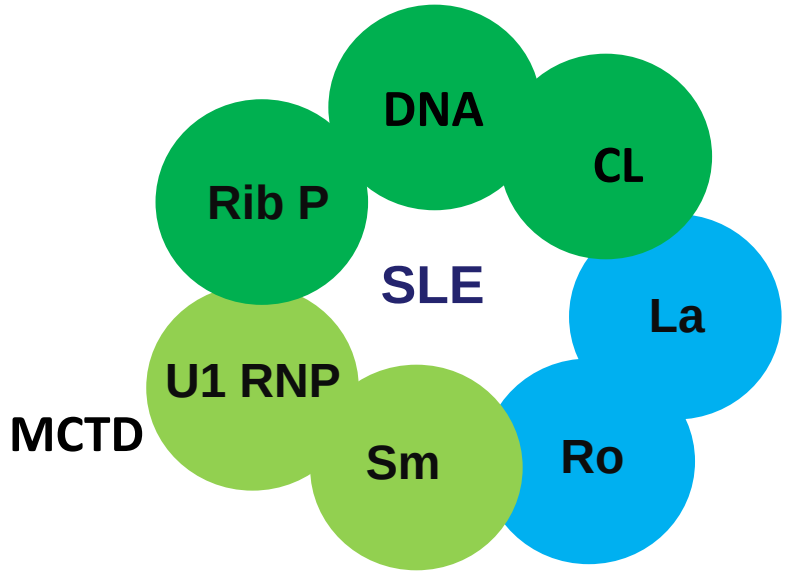




Autoantibodies in CTD



Autoantibodies in SLE



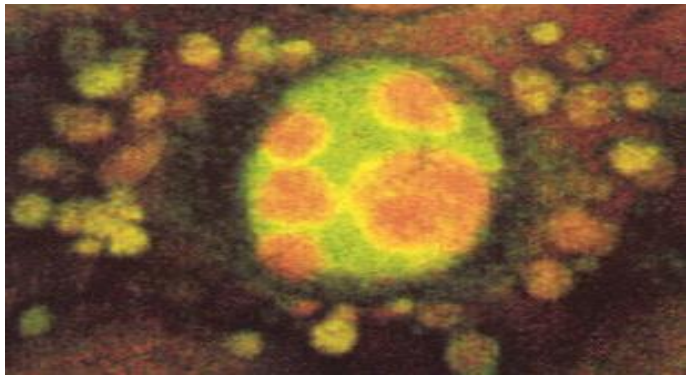
Autoantibodies in SLE

Autoantibody	Autoantigen
• Anti-ds-DNA	• Nucleosomes
• Anti-phospholipid	• Complex phospholipids
• Anti-Sm (U1RNP)	• snURPs
• Anti-Ro/La	• RNA-binding proteins
• Anti-C1q	• Early complement proteins
• Anti-ribosomal P	• Ribosomal proteins

Autoantigens Targeted in Systemic Lupus Erythematosus Are Clustered in Two Populations of Surface Structures on Apoptotic Keratinocytes

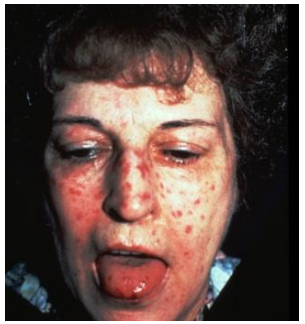
By Livia A. Casciola-Rosen,* Grant Anhalt,* and Antony Rosen‡

*From the Departments of *Dermatology, and ‡Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*



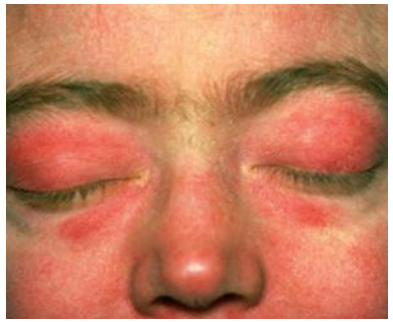
Autoantigens in surface blebs of UV irradiated keratinocytes

The spectrum of autoimmune connective tissue disease



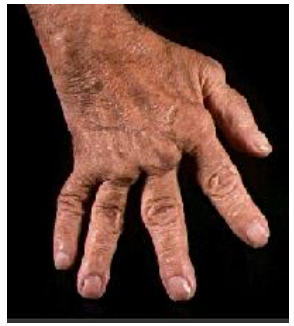
Scleroderma

Nucleolar RNP



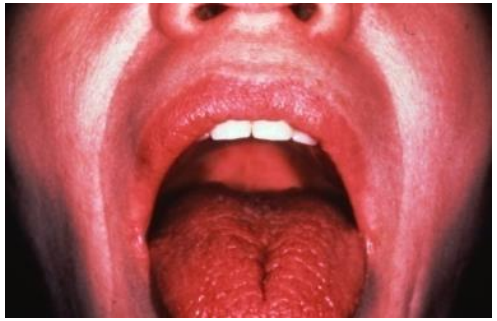
Dermatomyositis

Transcription factors
RNA synthetase



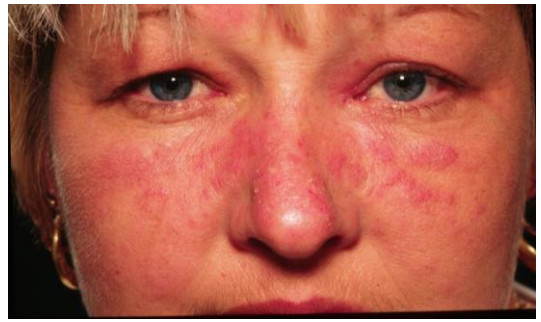
Rheumatoid arthritis

ACPA



Sjogren's

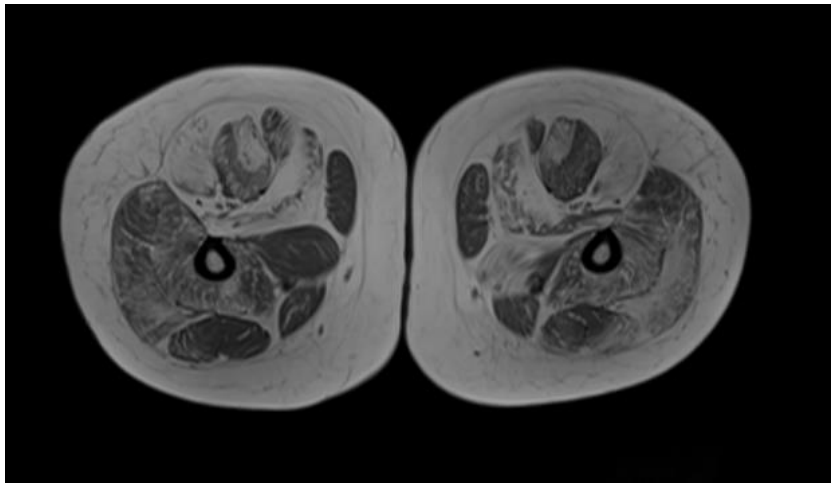
Ro/La (SS-A/SS-B)



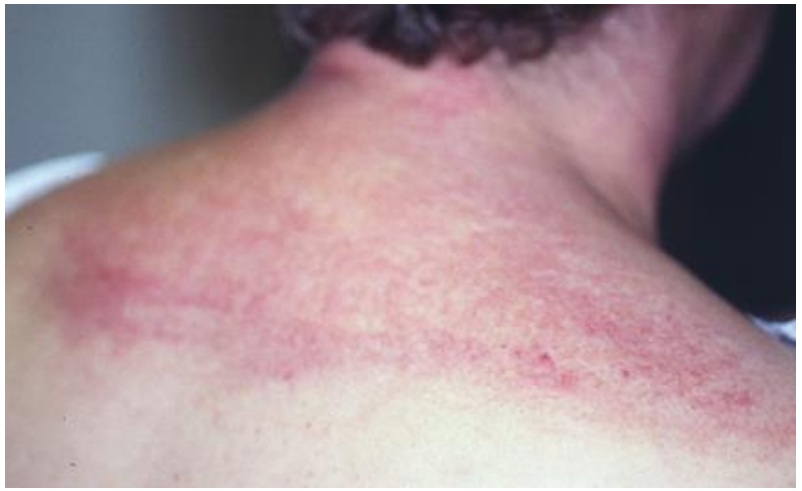
SLE

snRNPS
Nucleosome

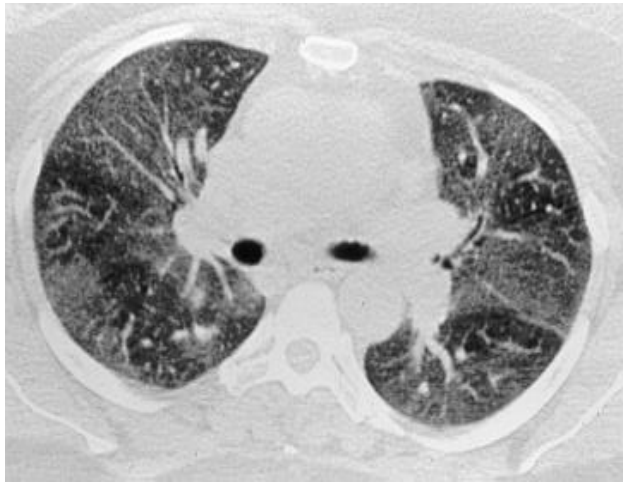
Dermatomyositis



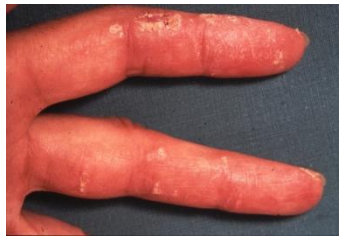
Muscle inflammation

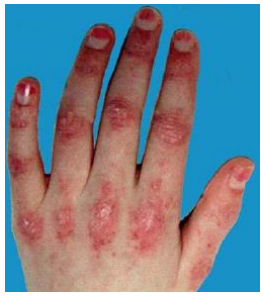


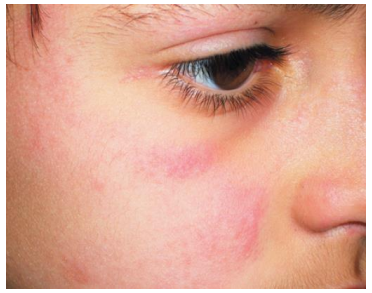
Skin disorder

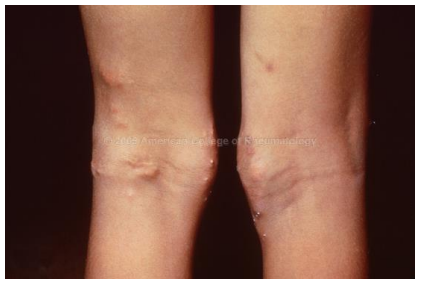


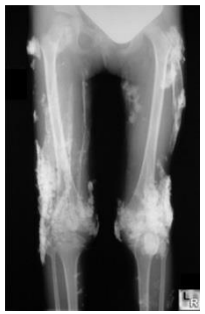
Interstitial lung disease



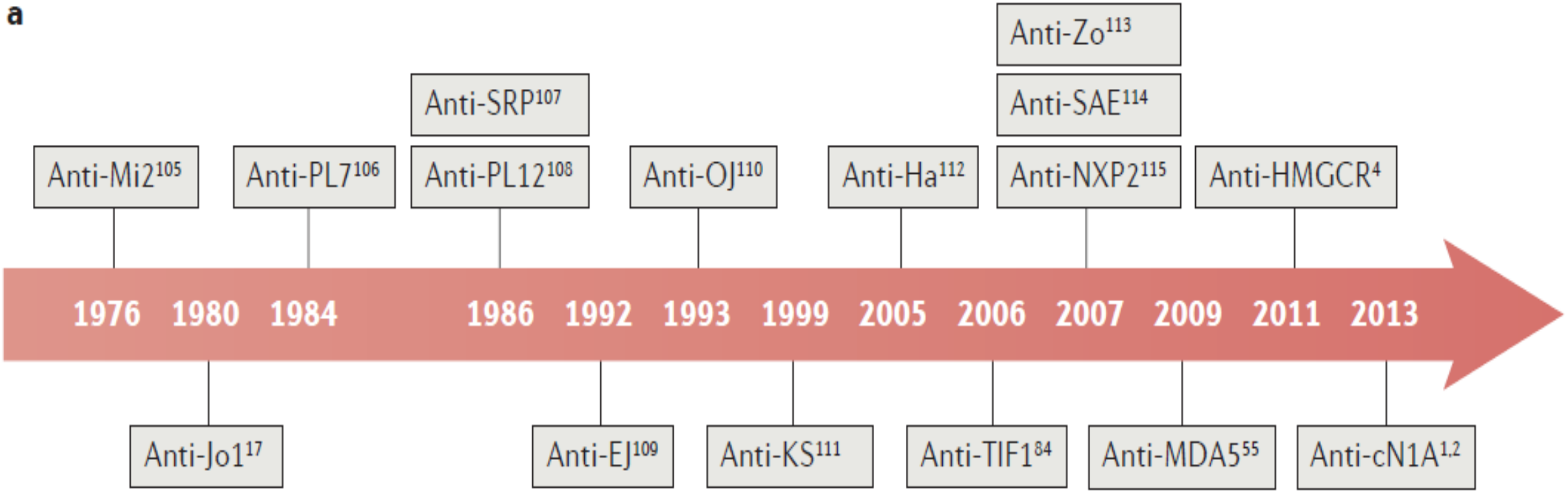








Timeline of myositis specific autoantibody discovery



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-TIF1-γ
  - Anti-NXP2
  - Anti-MDA5
  - Anti-HMGCR
  - Anti-cN-1A
- MAA (myositis associated autoantibodies)
  - Anti-PM-Scl
  - Anti-U1RNP
  - Anti-Ku
  - Anti-U3RNP
  - Anti-Ro (SSA)

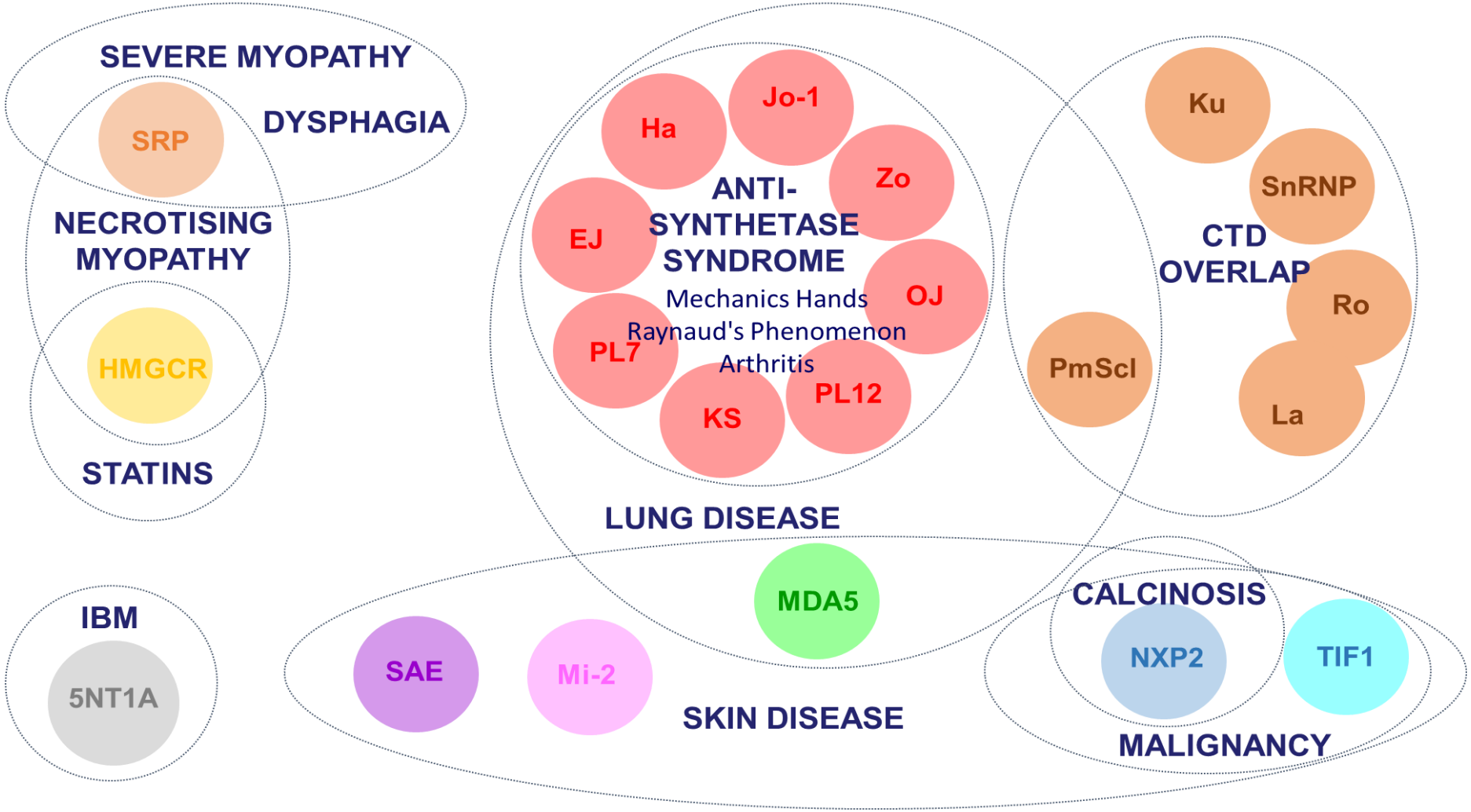
MSAs and target autoantigens I

Autoantibodies	Target autoantigen	Autoantigen function	Clinical phenotype
Anti-ARS Anti-Jo-1 Anti-PL-7 Anti-PL-12 Anti-EJ Anti-OJ Anti-KS Anti-Zo Anti-YRS	tRNA synthetase Histidyl Threonyl Alanyl Glycyl Isoleucyl Asparaginyl Phenylalanyl Tyrosyl	Intracytoplasmic protein synthesis Binding between an amino acid and its cognate tRNA	ASS Myositis Interstitial pneumonia Mechanics hands Arthritis Fever Raynauds
Anti-Mi-2	Helicase protein part of the NuRD complex	Nuclear transcription	Adult and juvenile DM Hallmark cutaneous disease
Anti-SRP	SRP 6 polypeptides and ribonucleoprotein 7SLRNA	Intracytoplasmic protein translocation (endoplasmic reticulum)	Severe necrotizing myopathy
Anti-HMGCR	3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase	Biosynthesis of cholesterol	Necrotising myopathy associated with statin use

MSAs and target autoantigens II

Autoantibodies	Target autoantigen	Autoantigen function	Clinical phenotype
Anti-p155/140	TIF1-γ	Nuclear transcription Cellular differentiation	Severe cutaneous disease in juvenile DM and cancer in adults
Anti-p140 (MJ)	NXP-2	Nuclear transcription (tumour suppressor gene p53)	Juvenile DM
Anti-SAE	SAE	Post-translational modification – targets include nuclear transcription factors	Adult DM May present with CADM first
Anti-CADM-140	MDA5	Viral RNA recognition	CADM Interstitial pneumonia
Anti-Mup44	Cytosolic 5'nucleotidase 1A (cN-1A)	Hydrolysis of AMP	Inclusion body myositis (Sjogren's)

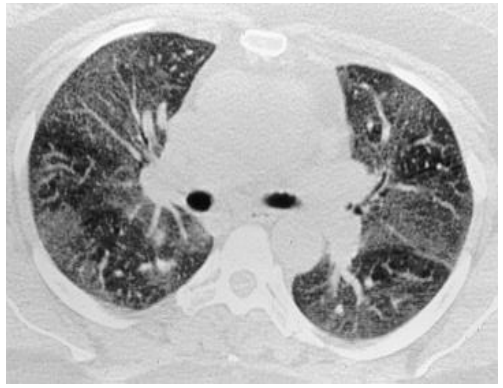
Myositis antibodies identify patterns of disease

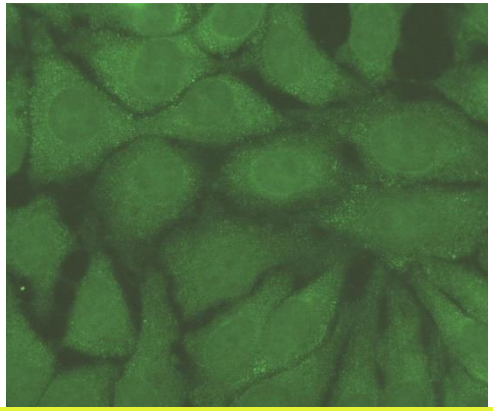


Betteridge and McHugh JIM 2015

Case A female born 1957

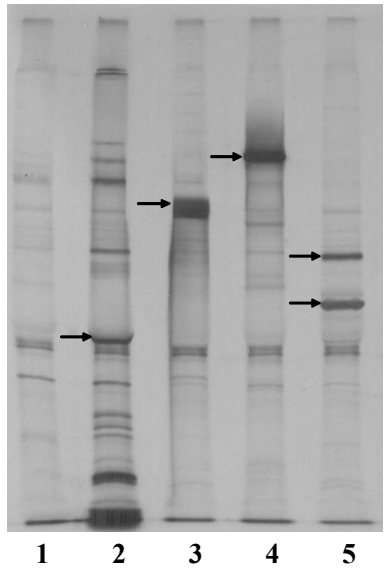
- 2006
- Breathlessness
- 6 months later
    - Proximal muscle weakness
    - Raynaud's
    - Arthralgia
    - Puffy fingers with some fissuring
- Invs
    - ANA weak positive
    - CK 9533 IU/L
    - HRCT non-specific interstitial pneumonia





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)

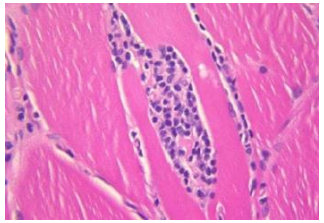


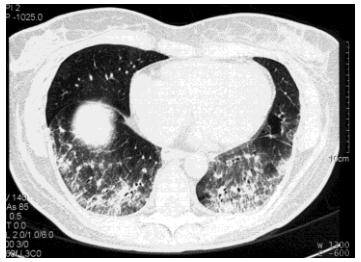
Protein Immunoprecipitation of bands at approximately 60 kDa and 70 kDa – phenylalanyl tRNA synthetase

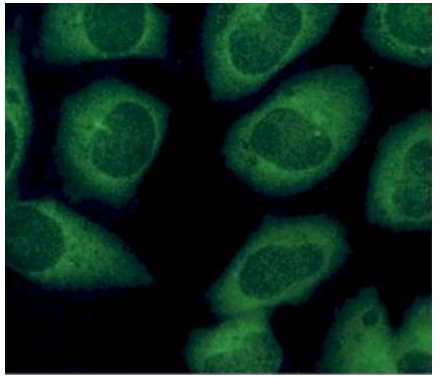
Anti-synthetase syndrome

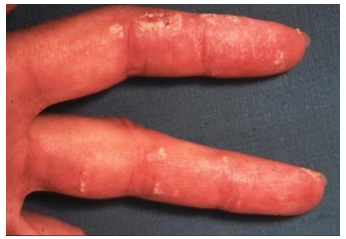
Clinical Features

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

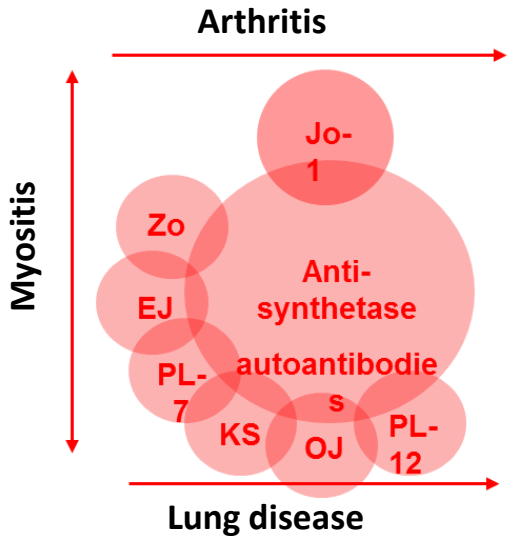


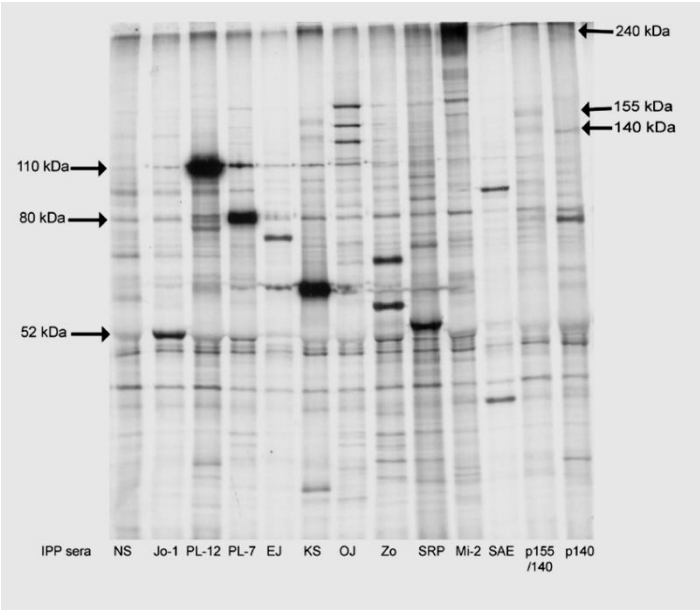






Autoantibody	tRNA synthetase target	Prevalence
Jo-1	Histidine	25-30%
EJ	Glycerine	<2%
PL-7	Threnyine	3-4%
KS	Asparigine	<2%
OJ	Isoleucine	<2%
PL-12	Alanine	3-4%
Zo	Phenylalanine	<2%



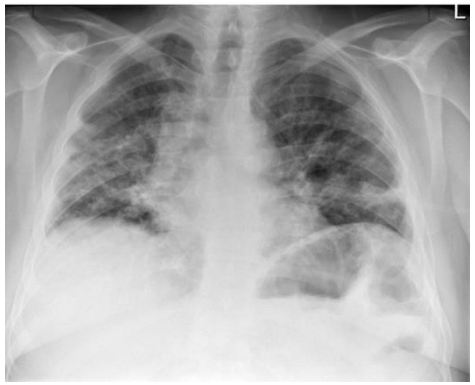


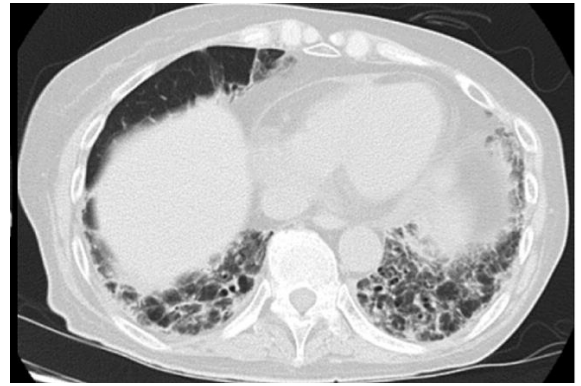
Key points regarding anti-synthetase syndrome

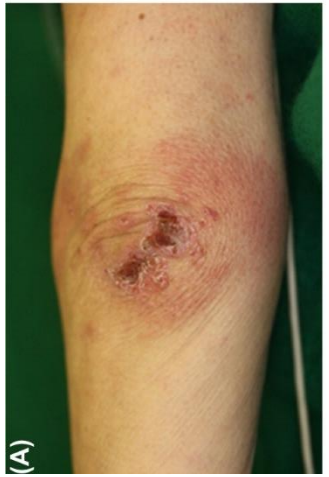
- Interstitial lung disease may be the predominant or even sole manifestation of myositis (anti-synthetase syndrome)
- Autoantibodies can be missed as they do not give a strong ANA on routine screening
- The additional presence of anti-Ro52 is associated with more severe ILD
- Uncommon in juvenile dermatomyositis (may relate to an association with smoking in adults)

Case B Chinese Female born 1960

- Admitted with fatigue, weight loss and ulcerative rash
- Rapidly progressive breathlessness
- No muscle weakness
- Invs
  - High Ferritin
  - Normal CK
  - Low O2 sats
  - PET normal
  - Anti-MDA5
- Diagnosis
  - CADM with RPILD

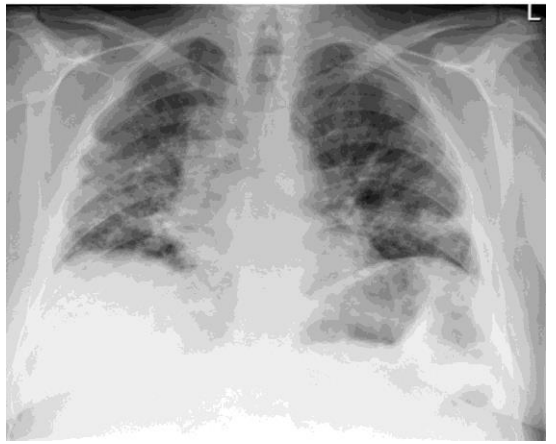


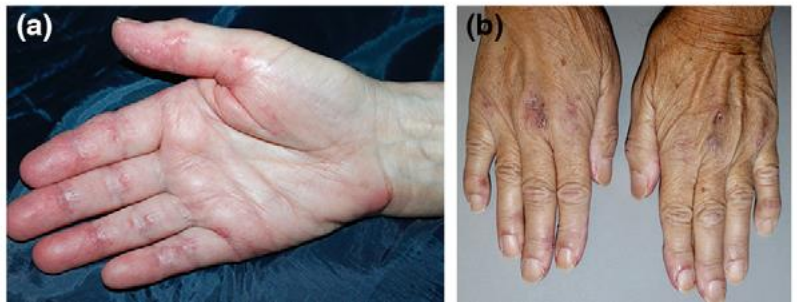




Anti-MDA5 Autoantibodies

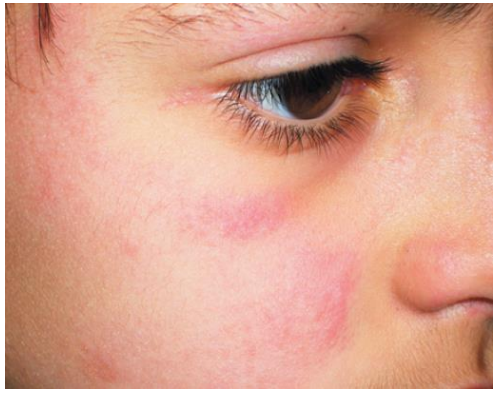
- More common in Asian myositis population (48%) than Caucasian (13%)
- In adults
  - Rapidly progressing ILD
  - Skin manifestations
    - Especially ulcerations (skin and mouth) and palmar papules
    - Other DM type rashes
- In children (7-38%)
  - Skin and oral ulcers
  - Milder muscle disease
  - ?ILD



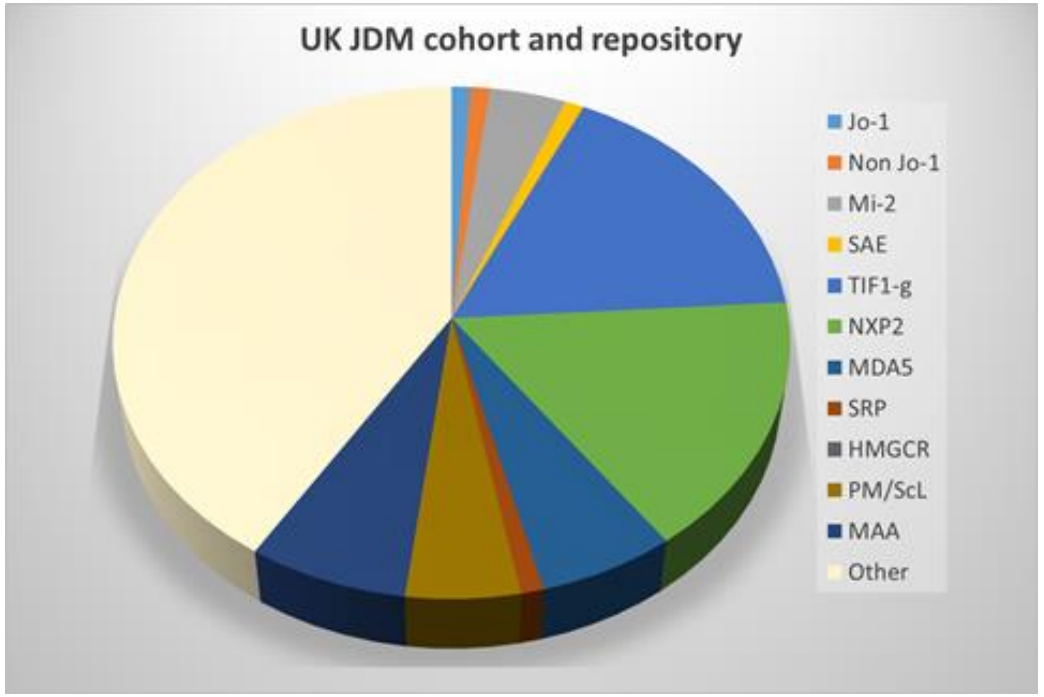


Fiorentino *et al* J Am Acad Dermatol 2011;65:25-34
Sato *et al* Arthritis Rheum 2005;52:1571-6
Nakashima *et al* Rheumatol 2010;49:433-40
Kobayashi *et al* J Pediatr 2011;158:675-7
Tansley et al Arthritis Res Ther 2014;16:R138
Moghadam et al Arthritis Care Res 2015

Autoantibodies in Juvenile MSD



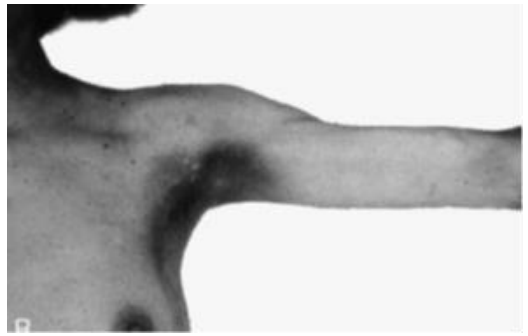
- MSDAs
  - Anti-TIF1g
  - Anti-NXP2
  - Anti-MDA5
  - Anti-Mi-2
  - Low frequency of anti-synthetase and anti-SRP
- MAAs
  - Overlap syndromes with scleroderma/lupus
    - Anti-PmScl
    - Anti-U1RNP

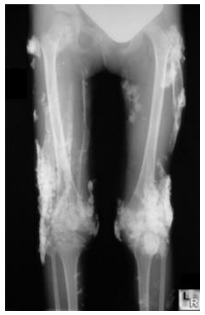


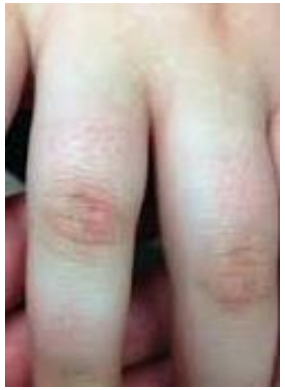
UK JDM Cohort and Biomarker study n = 347

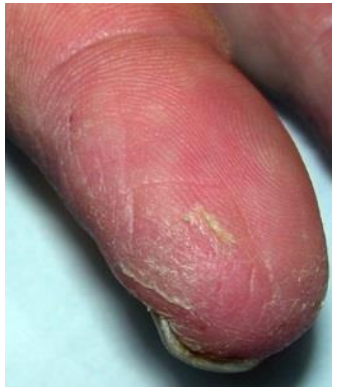
MDSAs in Juvenile MSD

- TIF1-γ
  - 17-33% of cases
    - More severe skin disease, ulceration, generalised lipodystrophy
- NXP2
  - 18-36% of cases
    - Calcinosis, contractures, muscle atrophy
- MDA5
  - 7-38% of cases
    - Skin and Oral Ulcers, Arthritis, Milder Muscle Disease, ILD
- Mi-2
  - 2-5% of cases
    - Milder disease course







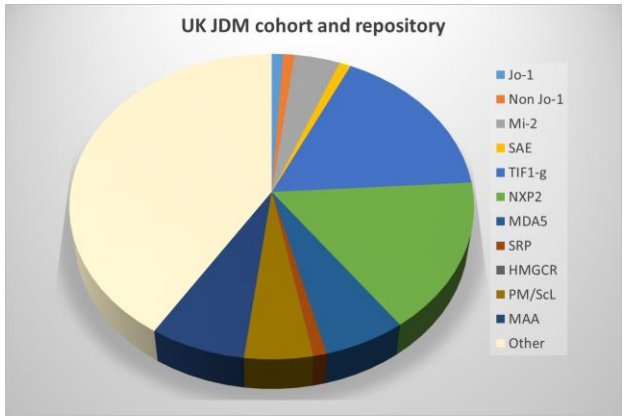


Bingham Medicine Baltimore 2008;87(2):70-86
Gunawardena Rheumatology 2008;47(3):324-8
Espada J Rheumatol 2009;36:2547-51
Rider Medicine Baltimore 2013 92(4) 223-43
Kobayashi *et al* J Pediatr 2011;158:675-7
Tansley Rheumatology 2014;53(12):2204-8
Tansley Arthritis Res Ther 2014;16:R138

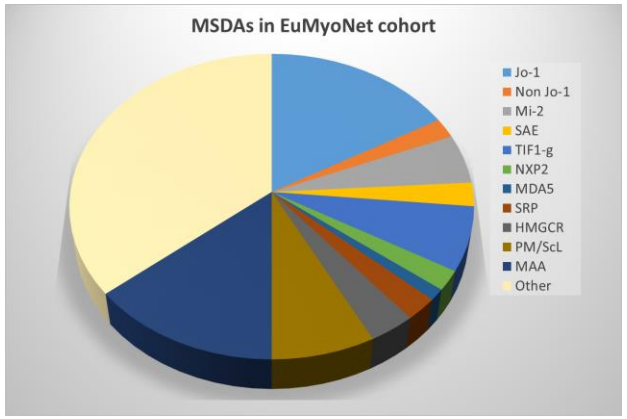
Patterns of Juvenile versus Adult MSD

- 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



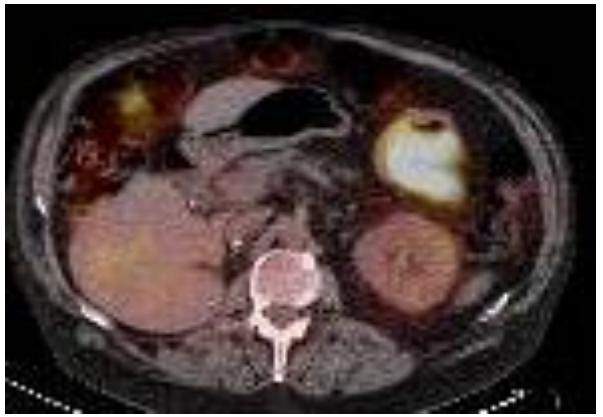
EUMYONET n = 1616

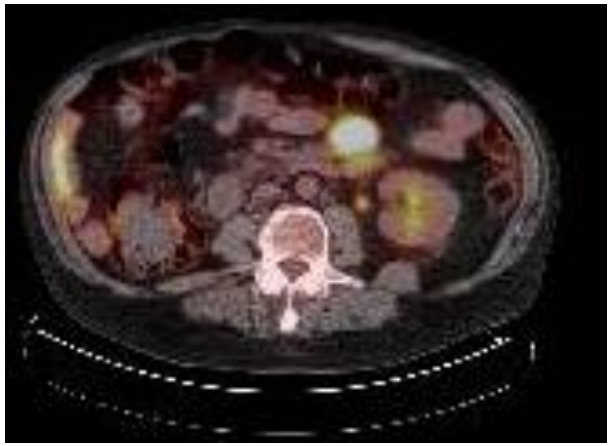
Myositis and cancer

- Association between cancer and myositis known for many years
- Risk of cancer 3 to 7 times higher in dermatomyositis
- Most common types of cancer
  - Ovary, lung, pancreas, stomach, colorectal, breast, lymphoma, nasopharynx (southeast Asians)
- The risk of malignancy development is highest within one year of myositis diagnosis
- Cancer-associated myositis (CAM) defined as concurrence of myositis and malignancy within 3 years
- Several studies have now shown that anti-transcriptional intermediary factor 1 is a myositis autoantibody and a risk factor for an associated cancer

Case C male born 1953

- **Acute admission 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
  - PET scan revealed recurrence of renal cell carcinoma
  - Anti-TIF1g positive





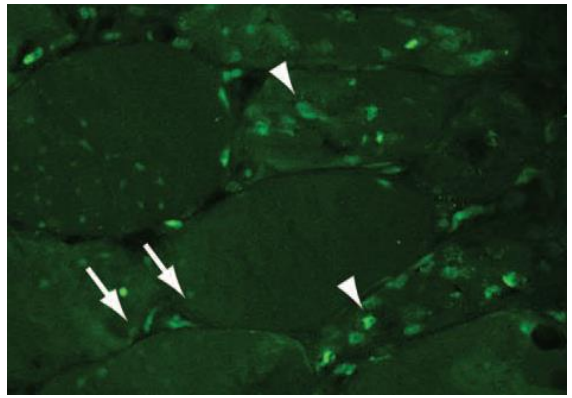
Transcriptional intermediary factor I
Clinical Associations of anti-TIF1 in EuMyoNet
(first 1616 cases – unpublished)

Clinical Feature	TIF1 Negative	TIF1 Positive	p value
Interstitial lung disease	31.2%	16.0%	=0.0038
Cancer (ever)	8.0%	32.2%	<0.0001
Cancer-associated myositis	2.3%	20.5%	<0.0001

Learning points from Case C

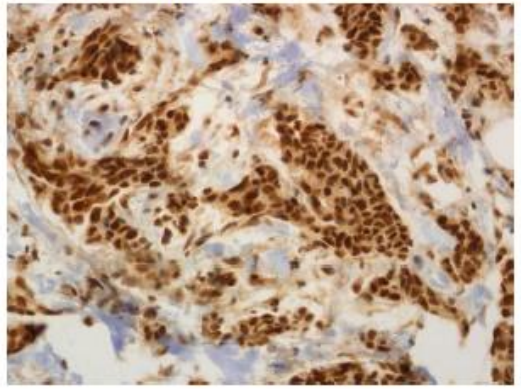
- A thorough screen for cancer is needed in dermatomyositis, especially with the presence of anti -TIF1γ (also anti-NXP2)
- The risk of cancer is also higher with age and in the presence of clinically amyopathic DM (CADM)

Cancerous cells may initiate an autoimmune reaction against muscle cells

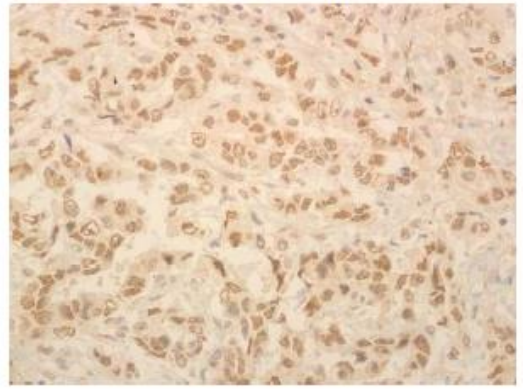


TIF1g is found in high levels within the nuclei of regenerating muscle fibres
Mohassel et al Arthritis Rheum 2015

Breast adenocarcinoma (IHQ 290)
anti-TIF1g positive CAM



Breast adenocarcinoma (IHQ 130)
Paired tumor from non-myositis patient

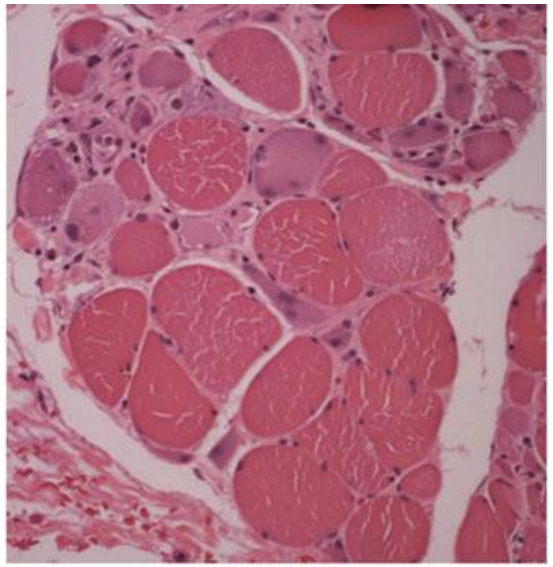


TIF1g a tumour suppressing protein is over-expressed in tumour cells from a CAM patient
Pinal-Fernandex et al Rheumatology 2018

Mutated or abnormally expressed protein becomes a target for an autoimmune reaction. In certain circumstances (e.g. regenerating muscle cells over-express myositis autoantigens) the anti-tumour response is directed towards skeletal muscle cells

Case E male born 1964

- 2006
  - Fatigue, weight loss, dysphagia, severe weakness, sclerodactyly
  - CK 14,000, EMG positive, Muscle biopsy necrotising myositis, serology anti-SRP
  - Slow response to Prednisolone and IVIG
  - MMDS 5
  - Anti-SRP positive
- 2007
  - Partial response to IV cyclophosphamide
  - Myocarditis, CK 1700, MMDS 17
  - Good response to rituximab and MMF
- 2008
  - Returned to work as self-employed builder, MMDS 30, CK 207
- 2009
  - Died of acute coronary event



Anti-SRP and Anti-HMGCR autoantibodies

Anti-SRP

Necrotizing Myopathy

- **Fatigue / Arthralgia**
- **Severe Weakness (Rapid Onset)**
- **High CK at Presentation**
- **Carditis**
- **Maybe refractory to standard treatments**
- **Titres may correlate with disease activity**

Targoff *et al* Arthritis Rheum 1990;33:1361-70

Wang *et al* NMD 2014;24:335-41

Benveniste *et al* Arthritis Rheum 2011;63:1961-71

Rider *et al* 2013, Medicine Baltimore 92(4) 223-43

Anti-HMGCR

Necrotizing Myopathy

- **Statin-Induced**
- **Not seen in patients on statins without myositis**
- **Raised CK**
- **Mild to severe weakness**
- **Anti-HMGCR levels correlate with indicators of disease activity**
- **Responsive to immunomodulatory treatment**

Christopher-Stine L *et al*. Arthritis Rheum. 2010:2757-66.

Mammen AL *et al*.. Arthritis Rheum 2011, 63: 713-21.

Mammen AL *et al*. Arthritis Care Res (Hoboken) 2012;64:269-72.

Werner JL *et al.* Arthritis Rheum. 2012;64:4087-93

Acknowledgements

Musculoskeletal Medicine Research Group

- Neil McHugh
- Anita McGrogan
- William Tillett
- John Pauling
- Alison Nightingale
- Rachel Charlton
- Julia Snowball
- **Zoe Betteridge**
- Hui Lu
- Philip Hamann
- **Sarah Tansley**
- Millie Green
- Richard Holland
- Victoria Flower
- Amel Badoume

- **University of Bath**
  - Amanda McKenzie
  - Mark Lindsay
  - Steve Ward
- **University of Exeter**
  - Gavin Shaddick (statistics)
- **University of Manchester**
  - Anne Barton, John Bowes (psoriatic arthritis)
  - **Hector Chinoy, Janine Lamb (myositis)**
- **University of Liverpool**
  - **Bob Cooper (myositis)**
- **University of Leeds**
  - Philip Helliwell (psoriatic arthritis)
  - Laura Coates (now Oxford) (psoriatic arthritis
  - Leeds CTU
- **UCL**
  - **Lucy Wedderburn (Juvenile myositis)**
- **University College Dublin**
  - Oliver FitzGerald (psoriatic arthritis)

Consortia

- British Isles Lupus Assessment Group
- **UK Myonet**
- **EuMyonet**
- Psoriatic Arthritis European Genetic Consortium (PAGE)
- Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA)
- PROMPT Study Group