

The Immunopathology of Sarcoidosis

Marc A. Judson, MD
Albany Medical College

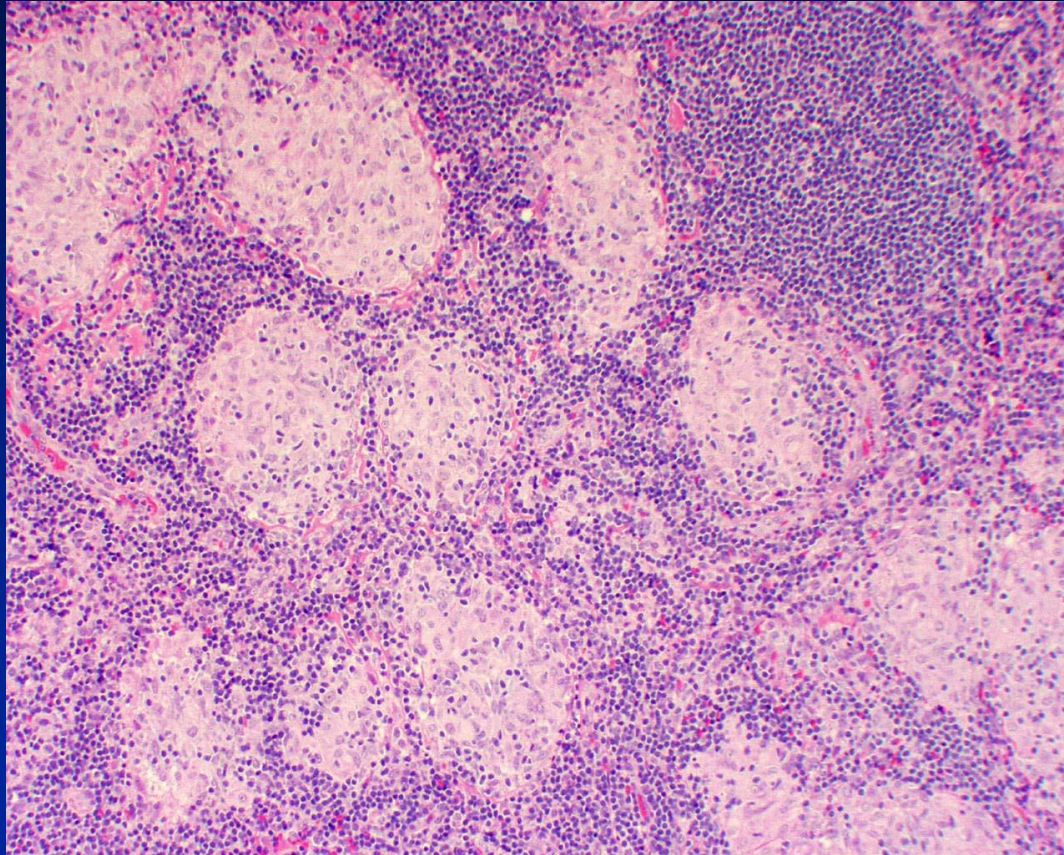
Disclosures

- Institutional grants
 - Mallinckrodt
 - Novartis
 - aTyr

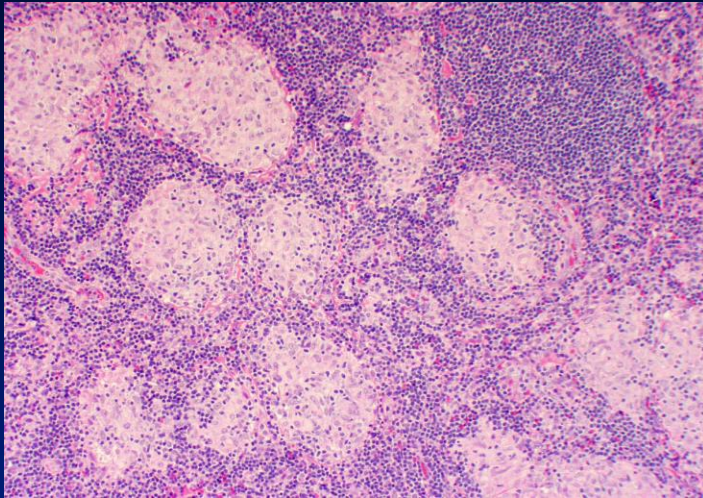
The immunopathology of sarcoidosis: learning objectives

- **Outline the mechanism of fibrosis formation in sarcoidosis**
- **List common parasarcoidosis syndromes**
- **List several potential putative antigens involved in the immunopathogenesis of sarcoidosis**

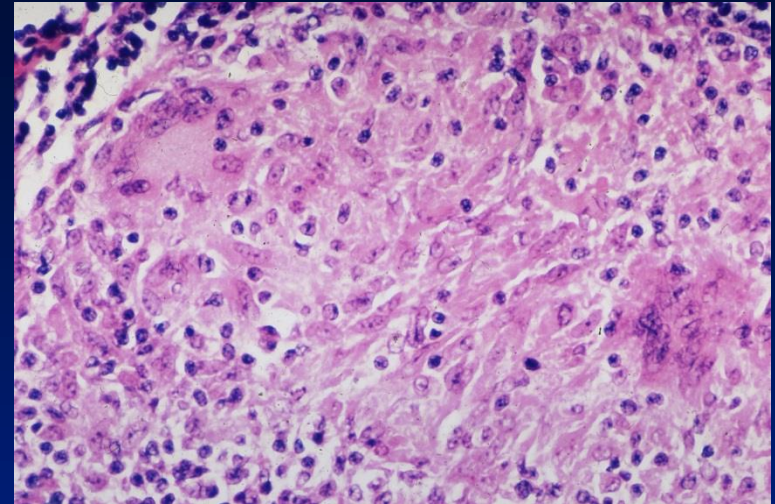
I: The Sarcoid Granuloma



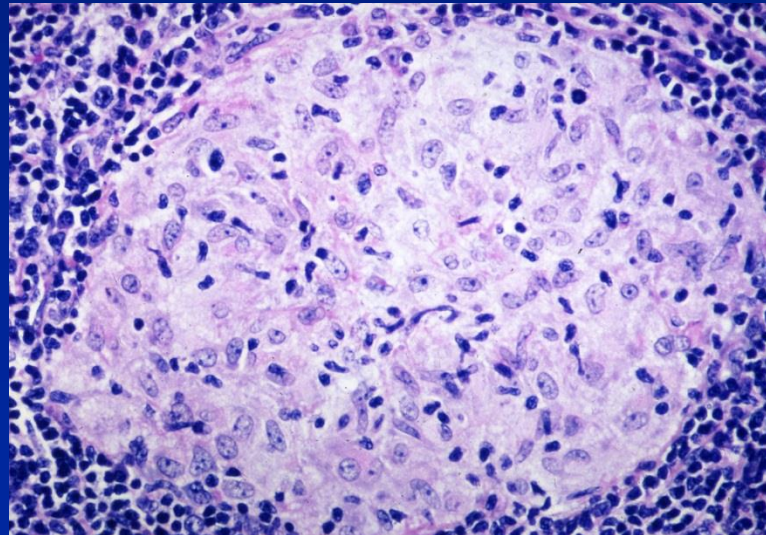
Pathology: The Sarcoid Granuloma



"Tight" granulomas



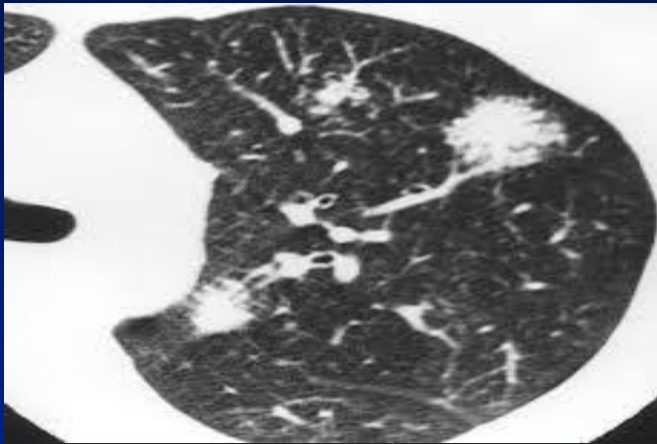
Multi-nucleated giant cells



Often relatively
non-necrotic

Surrounded by
lymphocytes

Granulomas are microscopic structures, but they tend to coalesce, forming larger nodules



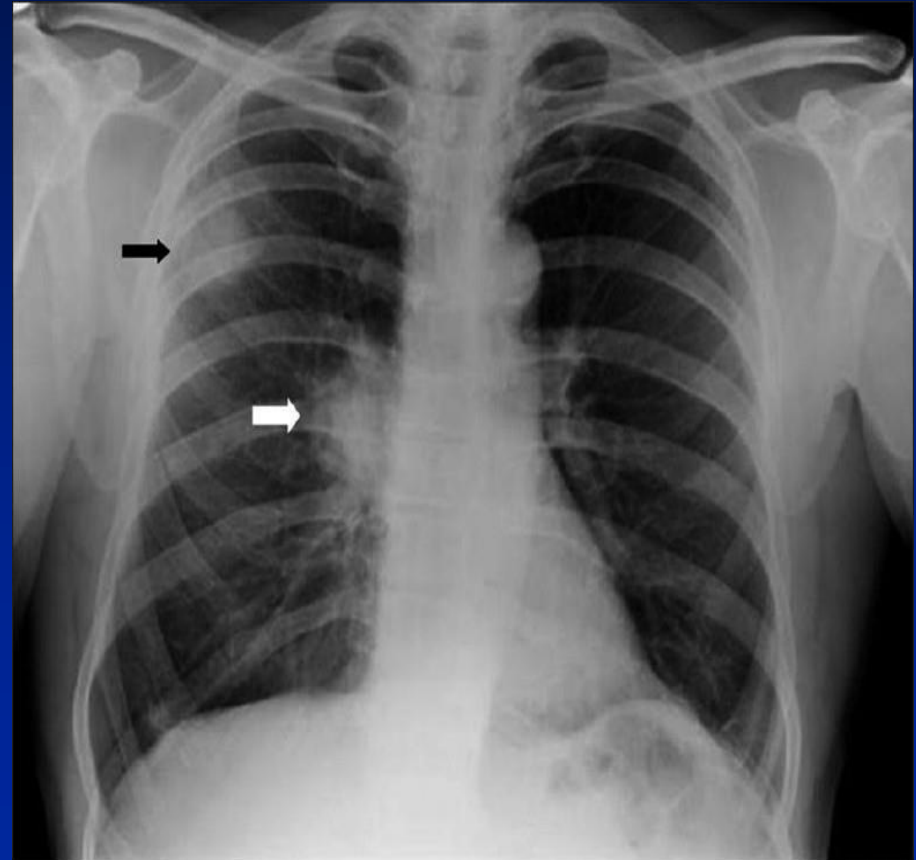
Galaxy sign



Sarcoidosis granulomas may cause minimal organ damage



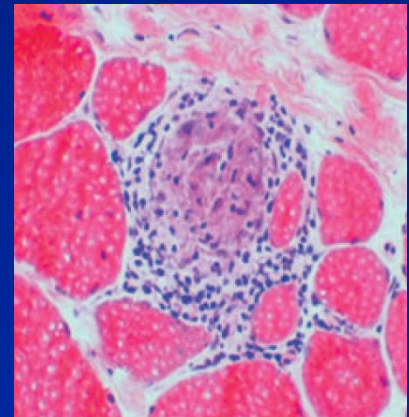
Sarcoidosis "potato" nodes



Lung cancer

Sarcoidosis granulomas may cause no/minimal organ damage

- **Neurosarcoidosis**
 - initial granulomatous meningitis
 - extension from the subarachnoid space via Virchow-Robin spaces¹
- **Kidney**
 - Interstitial nephritis²
- **Muscle**
 - CK often not elevated...only aldolase (connective tissue)³



1. Gullapalli D Curr Neurol Neurosci Rep 2004; 4:441
2. Bijol V. Intern J Surg Pathol 2006;14:57
3. Nozaki K. J Neurol Neurosurg Psychiatr 2009; 80:904

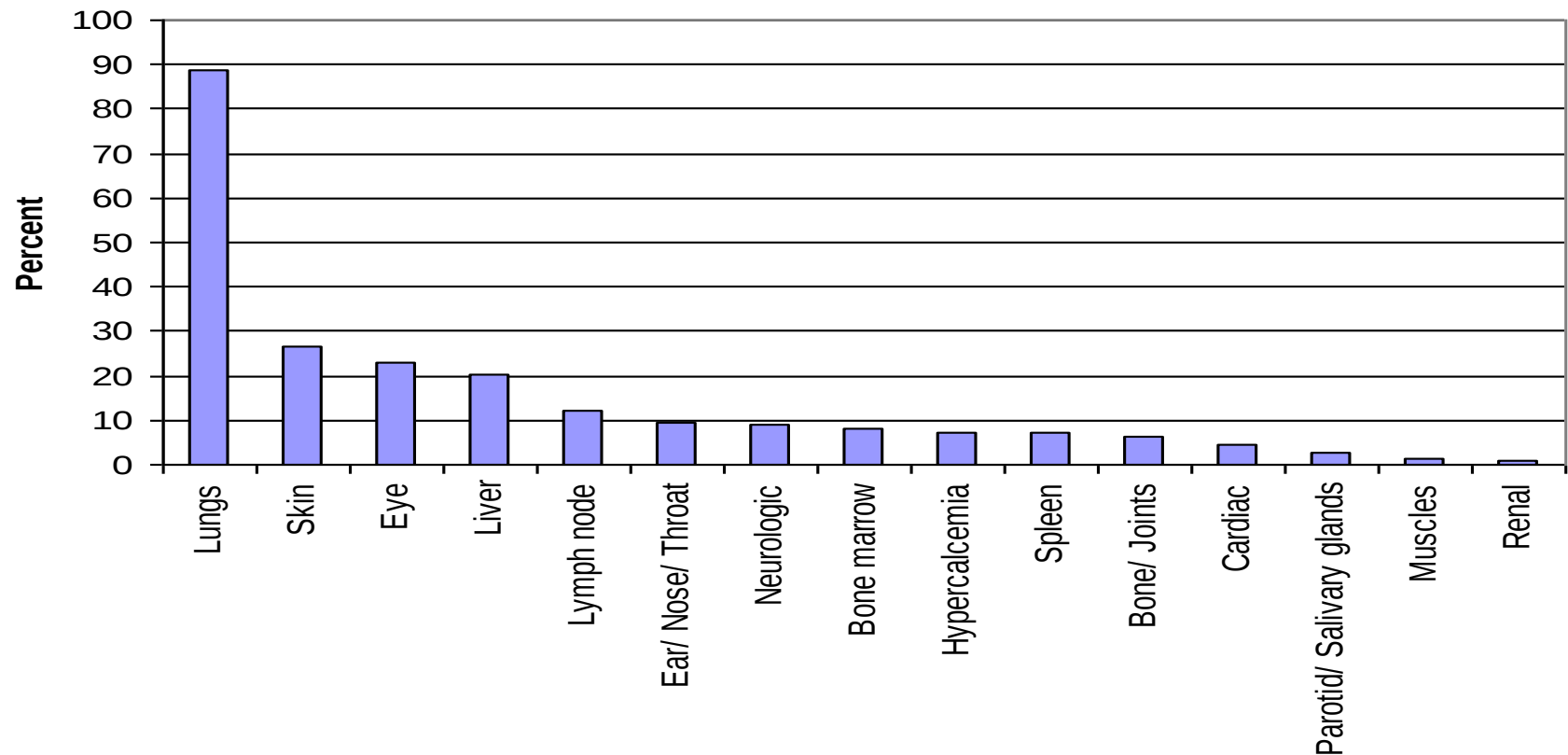
Sarcoid granulomas may cause significant organ damage

- **Infiltration of organ tissue**
 - Lung interstitium
- **Compression**
 - Vascular
 - Nerves
- **Expansion of tissues**
 - Liver
 - CNS

Sarcoid granulomas may develop in any organ

Percent of Organ Involved

N = 1582



Sarcoid granulomas usually cause organ damage slowly

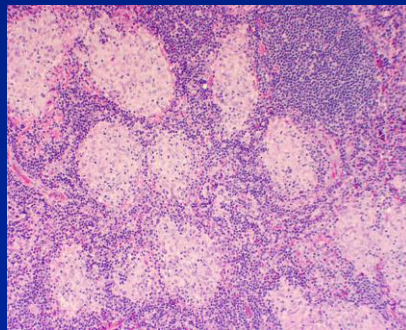
- It often takes weeks/months for granulomas to reach macroscopic size and cause significant symptoms¹
- **EXCEPTIONS**
 - Heart
 - arrhythmia / sudden death²
 - CNS

1. Moller DR. Eur Respir J. 2014; 44:1123

2. Koplan BA. Heart Rhythm 2006; 3:924

Sarcoid granulomas usually can resolve with or without therapy¹

GRANULOMA



+/- therapy

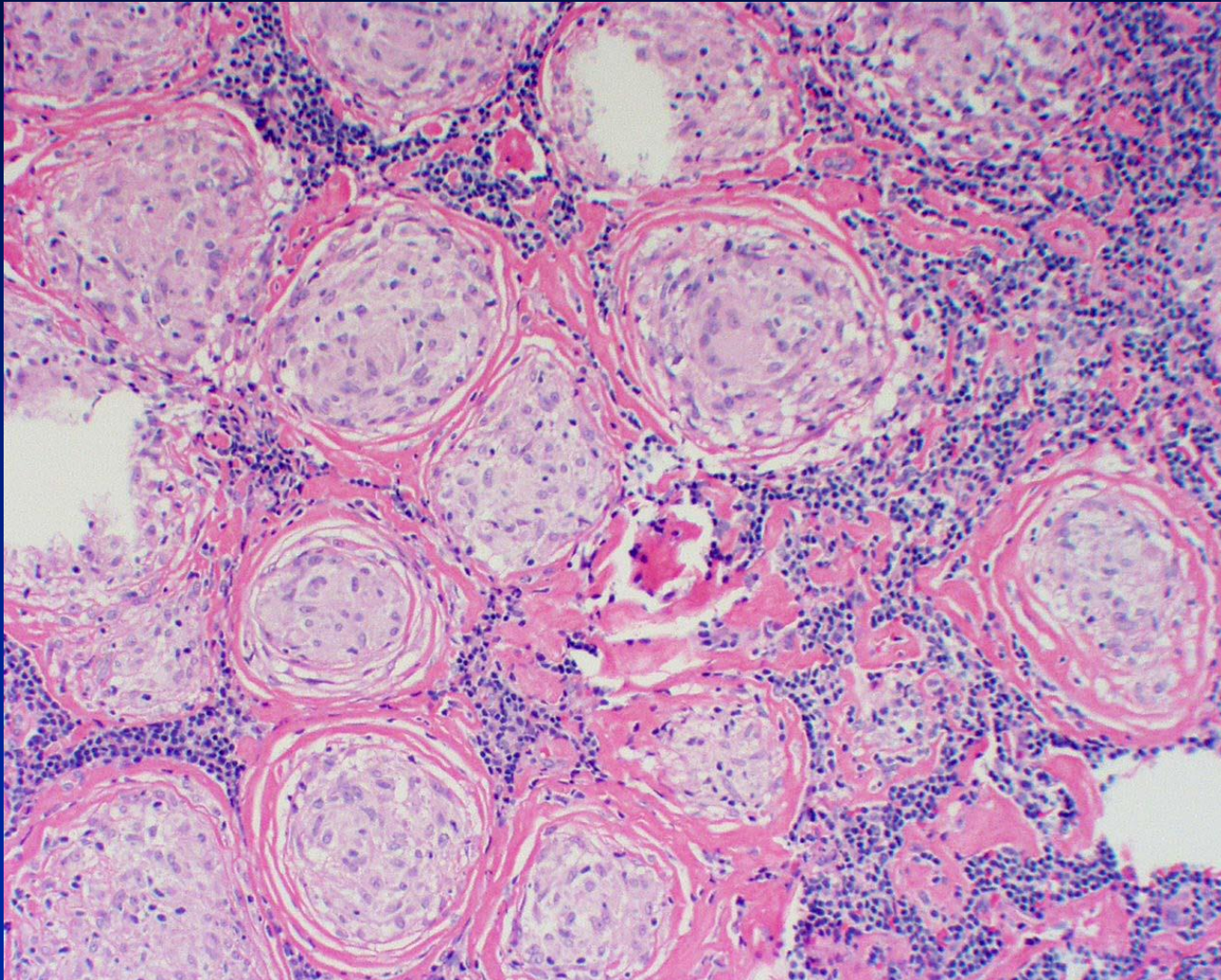


relapse

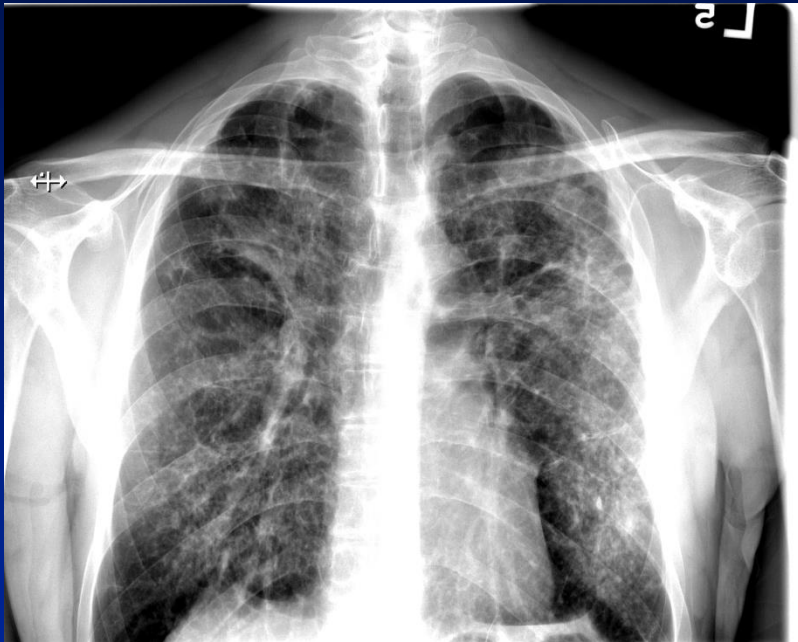
RESOLUTION

1. Gibson GJ. Thorax. 1996; 51:238

2: Fibrosis in sarcoidosis



Most serious and permanent damage from sarcoidosis is related to fibrosis

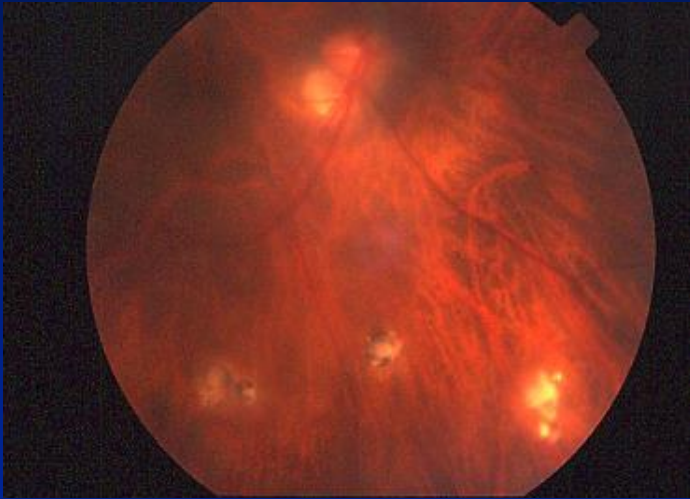


- most severe pulmonary dysfunction¹
- most/all pulmonary deaths^{2,3}
- most/all mycetoma⁴
- most pulmonary hypertension⁵

Stage 4 fibrocystic sarcoidosis

1. Patterson KC. Ann Am Thorac Soc. 2013; 10:362
2. Viskum K. Eur Respir J 1993; 6:349
3. Reich JM Chest 2002; 121:32
4. Hours S. Medicine 2008; 87:142
5. Handa T. Chest 2006; 129:1246

Most serious and permanent damage from sarcoidosis is related to fibrosis

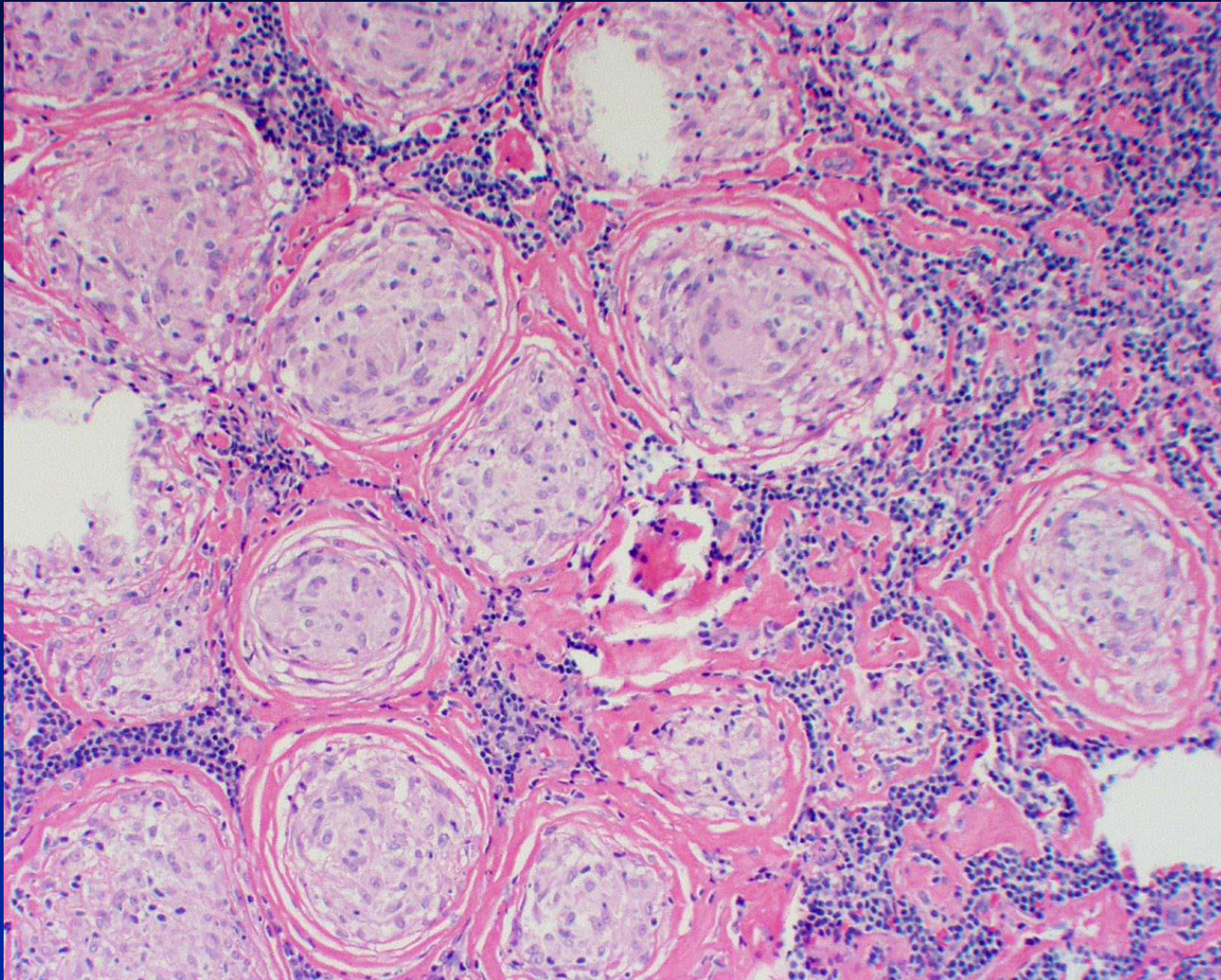


chorio-retinal scar



scar from skin sarcoidosis

Chronic sarcoidosis: active fibrosis plus scarring



Chronic sarcoidosis: active fibrosis plus scarring

- 22/26 (85%) of fibrotic pulmonary sarcoidosis patients had + lung PET uptake¹

1. Mostard RL. Respir Med. 2013; 107:439

Cardiac sarcoidosis: active disease plus scar

FDG PET scan findings:

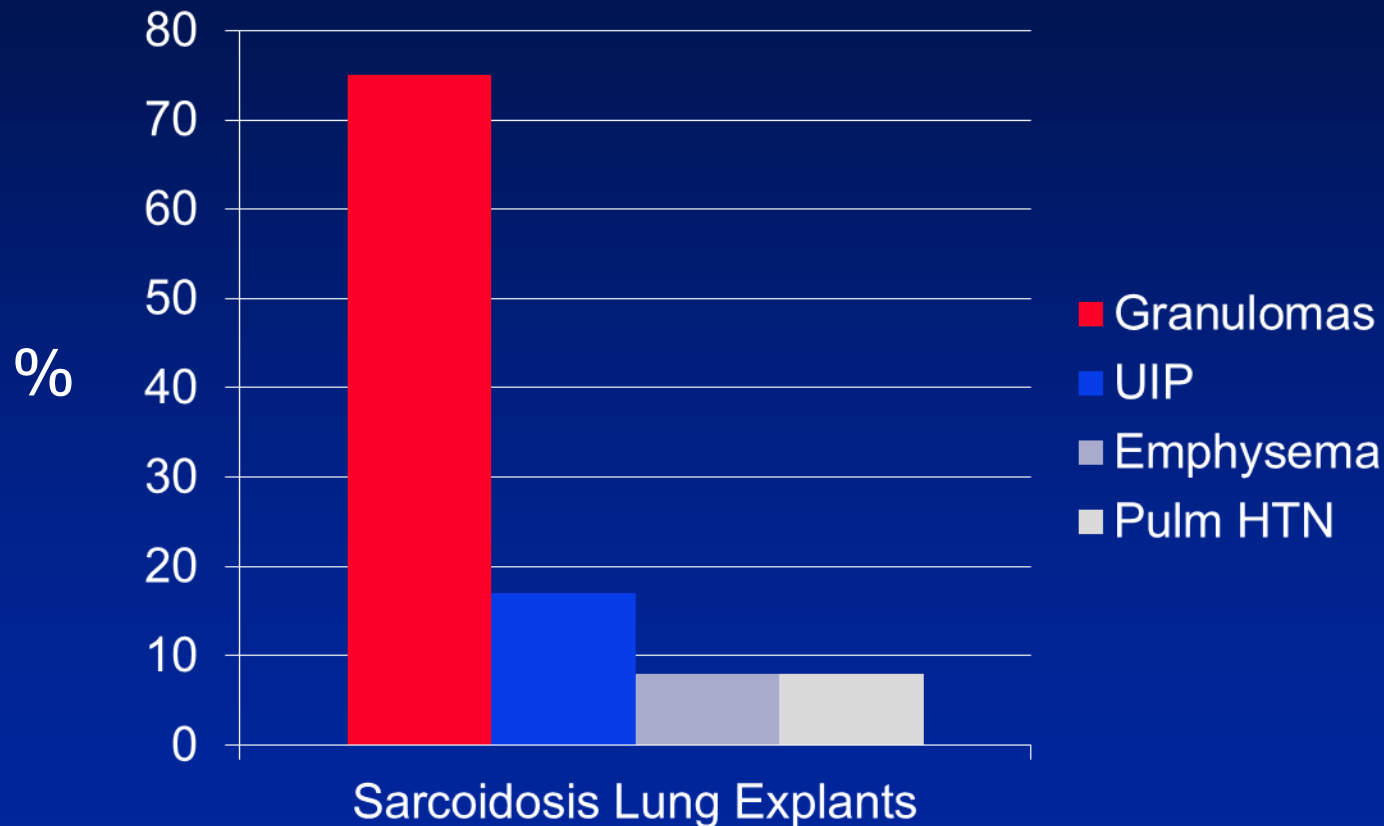
Abnormal perfusion and metabolism					
Positive	Focal increase ("mismatch pattern")	23 (19%)			Presence of active inflammation ± scar in the same location
Positive	Focal on diffuse	6 (5%)			Similar to above but also areas of inability to suppress FDG from normal myocardium vs. diffuse inflammation
Positive	Focal increase (different area)	5 (4%)			Presence of both scar and inflammation but in different segments

Blankstein R. J Am Coll Cardiol 2014; 63:329-336

- Ventricular ectopy in sarcoidosis
 - Re-entry around areas of scar¹
 - Promoted by active inflammation²

1. Stees CS. J Cardiovasc Electrophysiol. 2010 June 17
2. Tselentakis EV. J Surg Res. 2006; 135:68

Fibrosis in sarcoidosis is thought secondary to the granulomas



Zhang C. Chest 2016; 149:499

Progression of active pulmonary sarcoidosis

Worse
spirometry
and
symptoms

Normal
spirometry
And no
symptoms



Progression of active pulmonary sarcoidosis: non-fibrotic phenotype

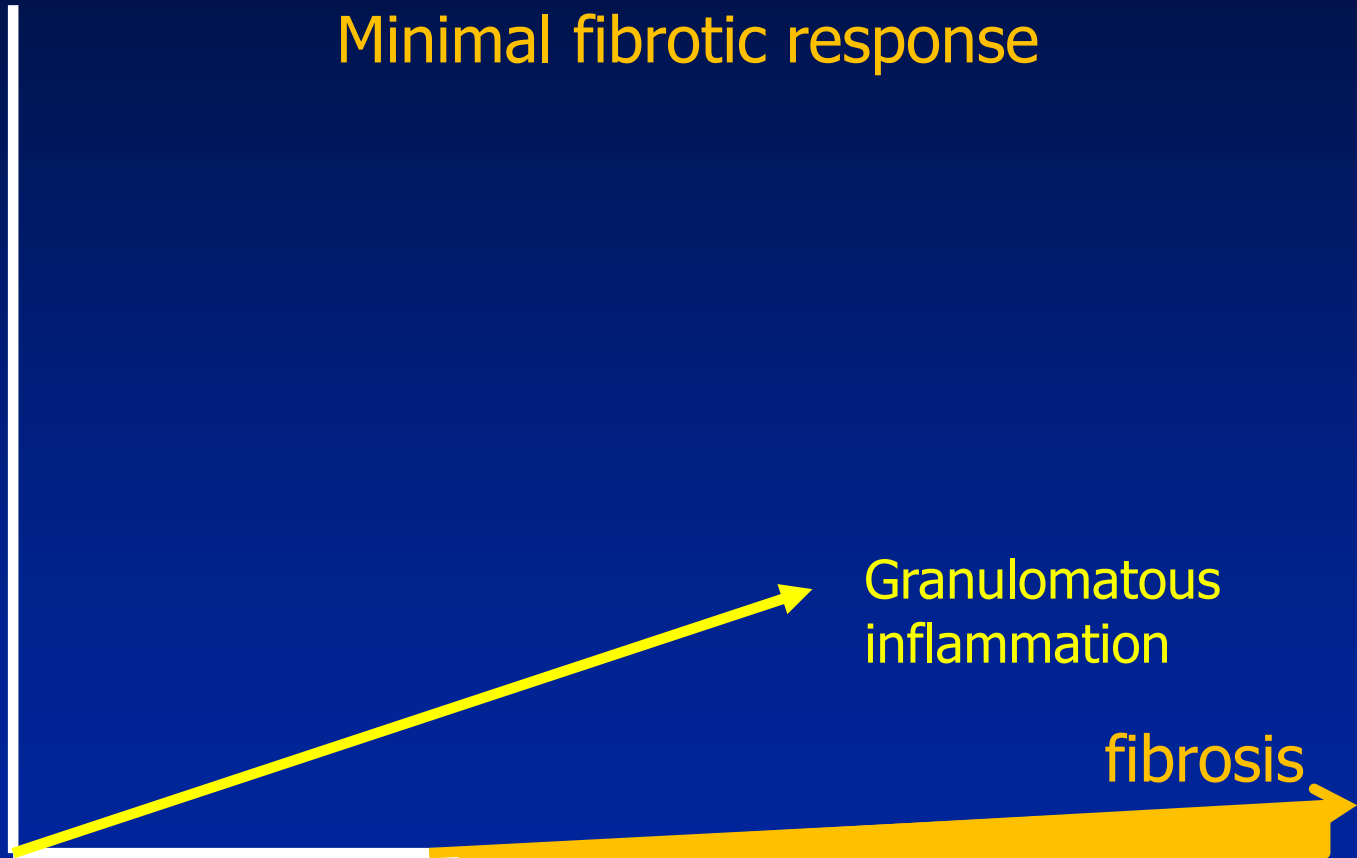
Worse
spirometry
and
symptoms

Minimal fibrotic response

Normal
spirometry
And no
symptoms

Granulomatous
inflammation

fibrosis



Progression of pulmonary sarcoidosis: fibrotic phenotype

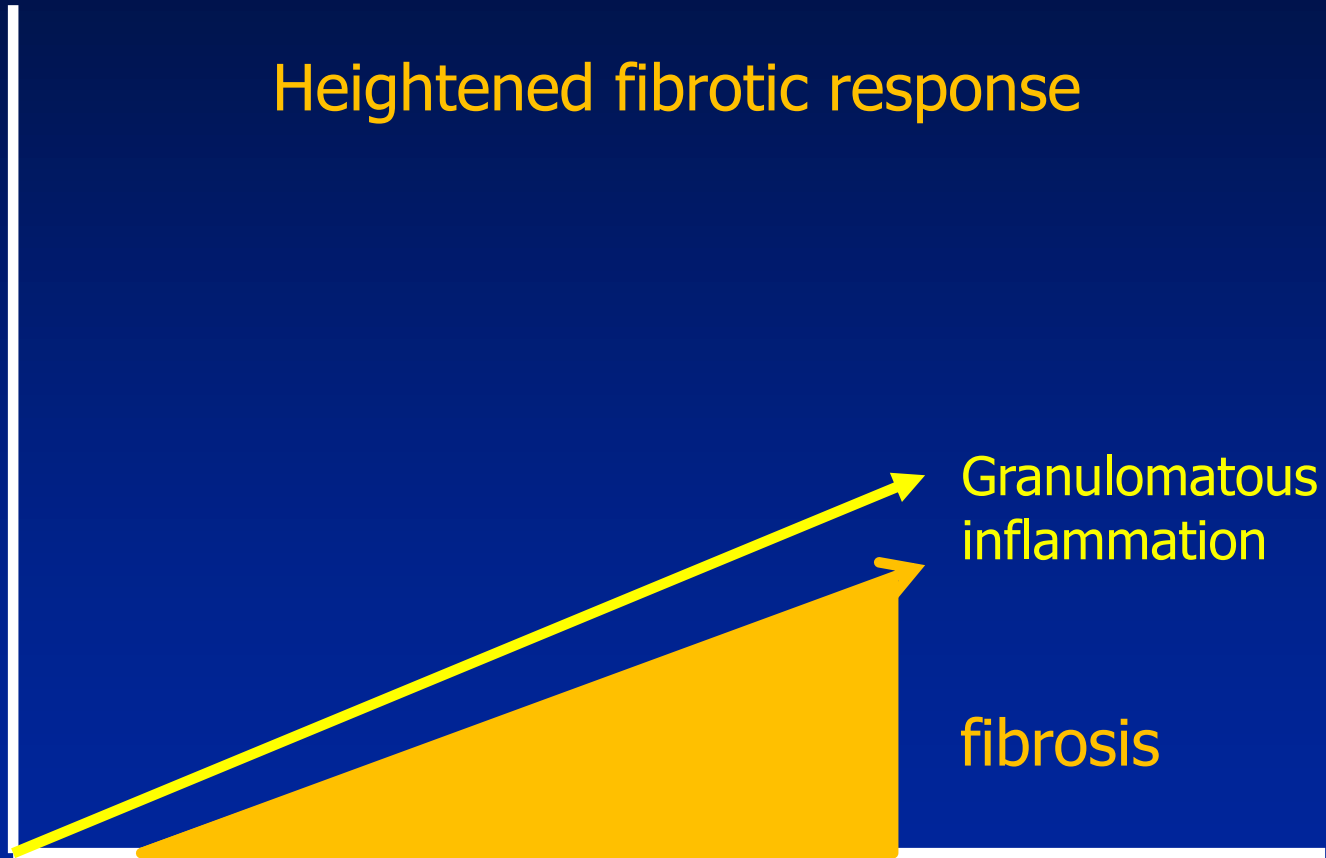
Worse
spirometry
and
symptoms

Heightened fibrotic response

Normal
spirometry
And no
symptoms

Granulomatous
inflammation

fibrosis



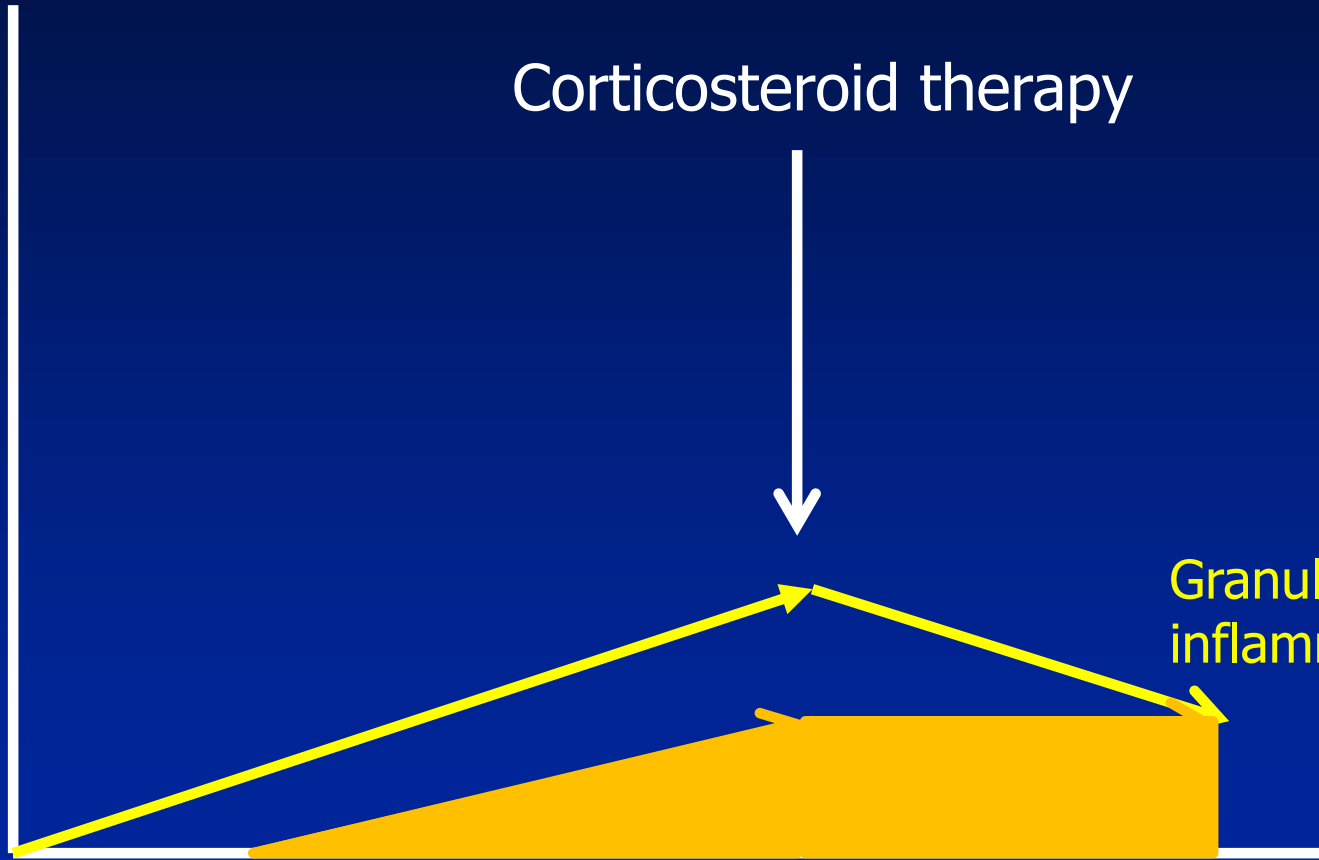
Treatment of active sarcoidosis

Worse
spirometry
and
symptoms

Corticosteroid therapy

Normal
spirometry
And no
symptoms

Granulomatous
inflammation
fibrosis



The patients we WANT in clinical sarcoidosis trials

Worse
spirometry
and
symptoms

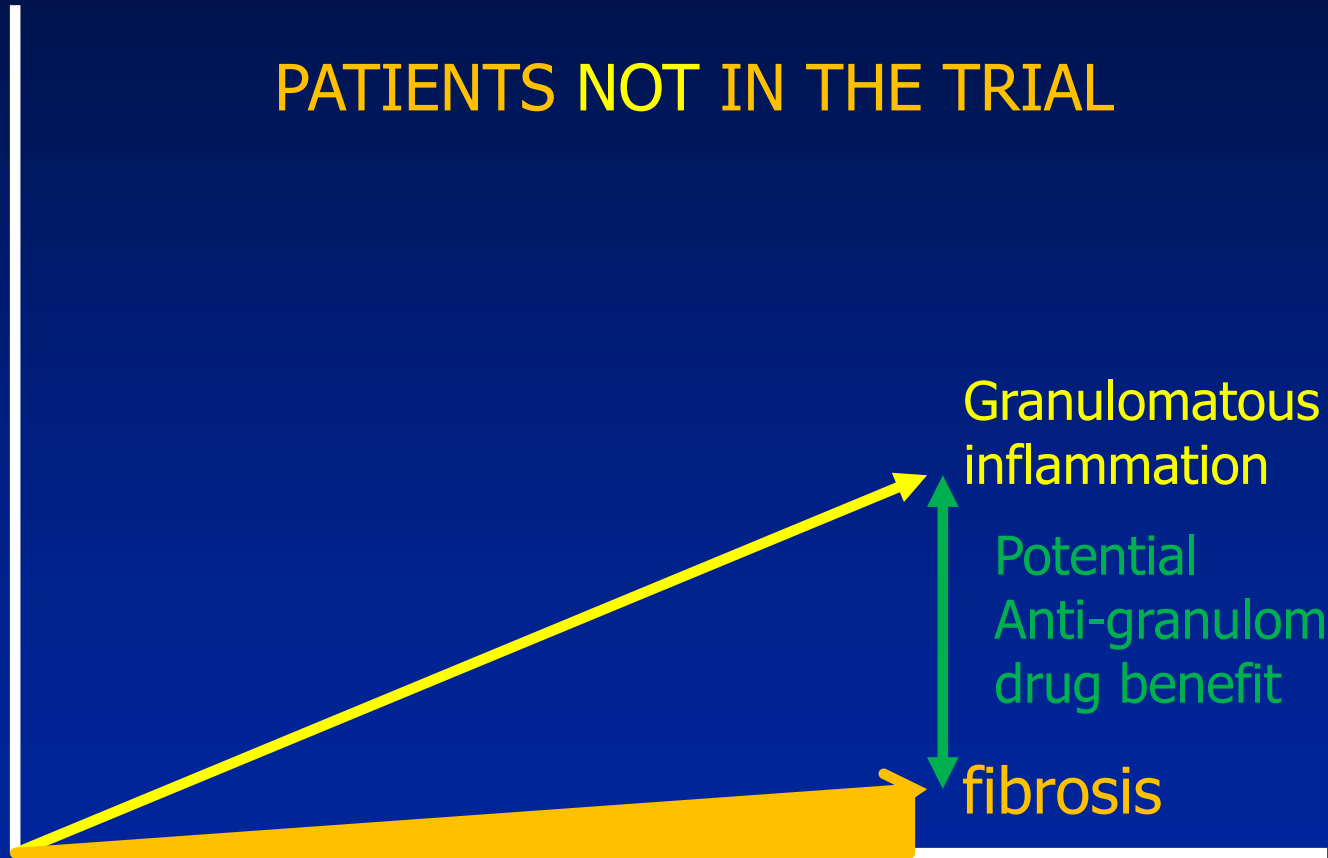
PATIENTS NOT IN THE TRIAL

Normal
spirometry
And no
symptoms

Granulomatous
inflammation

Potential
Anti-granulomatous
drug benefit

fibrosis



The patients we GET in clinical sarcoidosis trials

Worse
spirometry
and
symptoms

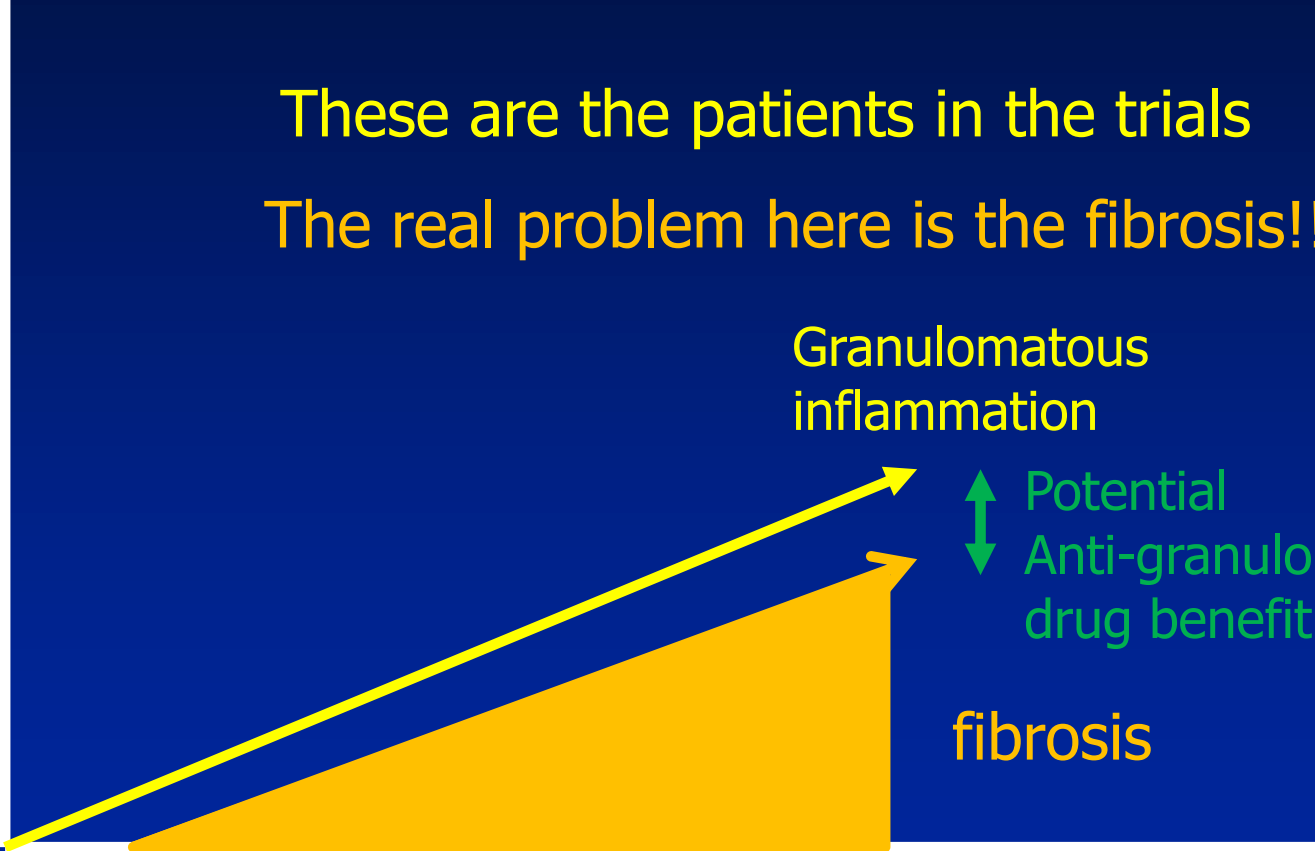
These are the patients in the trials
The real problem here is the fibrosis!!!

Normal
spirometry
And no
symptoms

Granulomatous
inflammation

Potential
Anti-granulomatous
drug benefit

fibrosis



The major clinical problem in sarcoidosis is fibrosis

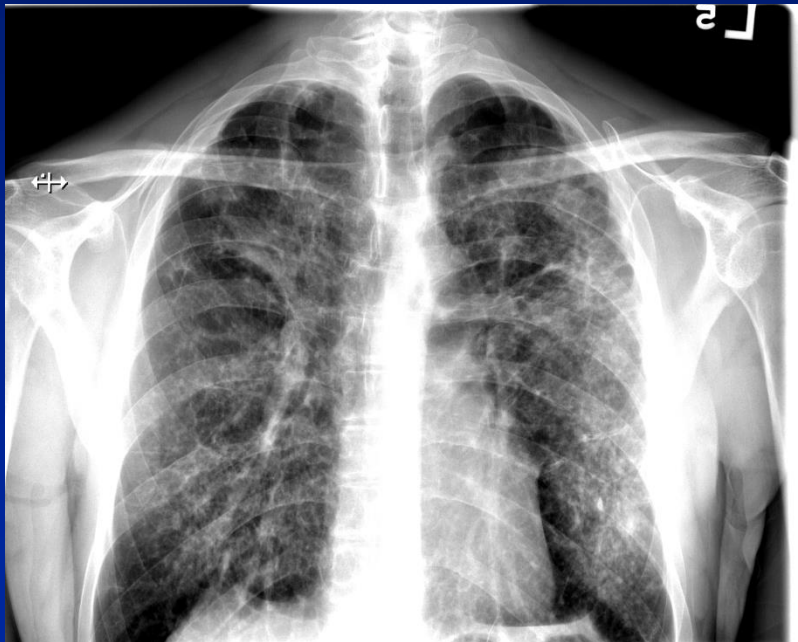
- Is the scarring all from granulomas?
- Is the scarring related to the host?
- **ALMOST NO WORK ON:**
 - Identifying those at risk of fibrosis
 - Treatment intervention trials

The major clinical problem in sarcoidosis is fibrosis

Current sarcoidosis drug treatment trials:

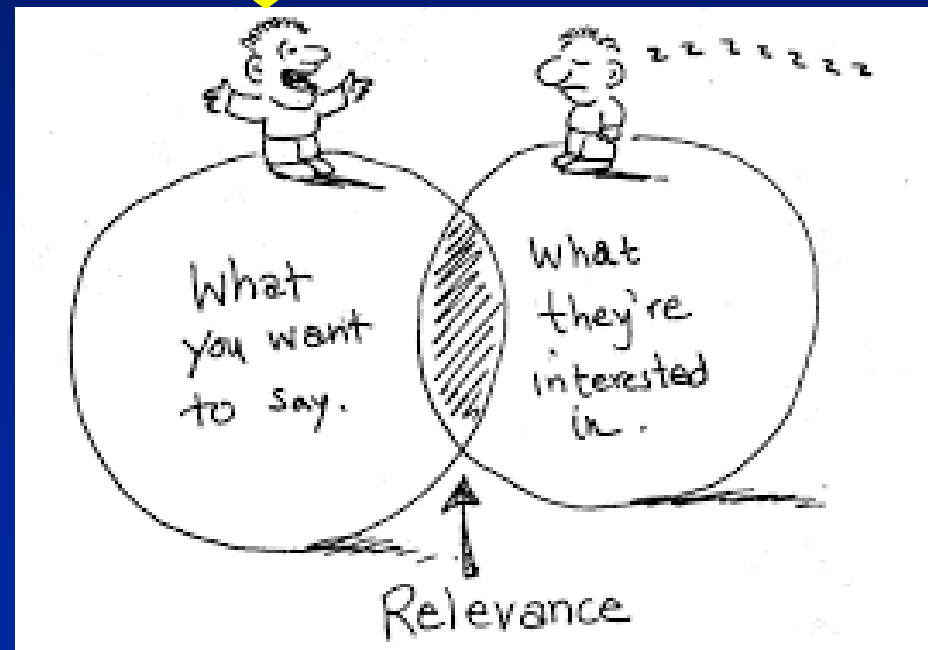
Current screening technique:

FVC = 61% pred



I've got a
GREAT
anti-granuloma
drug!!!

I'm fibrosed



Potential Biomarkers to Screen for Fibrotic Sarcoidosis I

- SNP of GREM 1¹
 - encodes for gremlin that is a secreted glycoprotein and member of the bone morphogenetic proteins
 - OR of fibrocytic sarcoidosis > 6.0
 - SNP of Card15 (caspase recruitment domain), AKA (NOD)2 nucleotide oligomerization domain²
 - Associated with fibrocystic disease and worse FEV1 at 4 yrs
- 1: Heron. M Tissue Antigens 2011; 77:112
2: Manouvrier-Hanu. Am J Med Genet 1998; 61:219

Potential Biomarkers to Screen for Fibrotic Sarcoidosis II

- Allele of the TGF- β 3 isoform¹
 - Associated with fibrotic sarcoidosis
- SNP rs35705950
 - a promoter polymorphism for the Mucin 5B gene
 - Strongly associated with IPF^{2,3}
 - **NOT** associated with fibrotic sarcoidosis⁴

1: Kruit A. Chest 2006; 129:1584

2: Seibold MA. NEJM 2011; 364:1503

3: Zhang Y. NEJM; 2011:1576

4: Stock CJ. Thorax 2013; 68:436

Sarcoid granuloma-fibrosis interaction

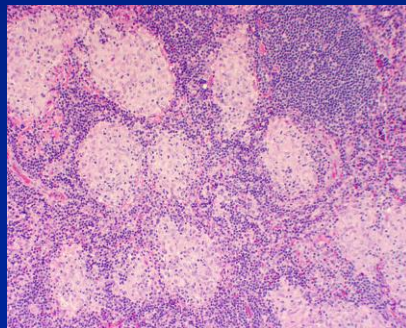
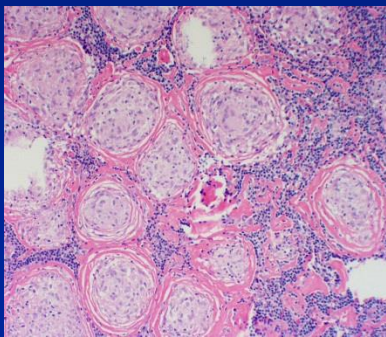
Pre-granulomatous inflammation

???



FIBROSIS

GRANULOMA



+/- therapy



relapse

RESOLUTION

3: Parasarcoidosis

Symptoms and/or dysfunction in sarcoidosis
not directly related to deposition
of sarcoid granulomas

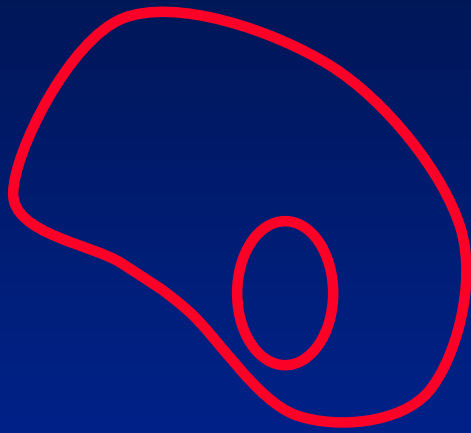
They may not respond to anti-granulomatous therapy

Parasarcoidosis syndromes

- Vitamin D dysregulation
- Erythema nodosum
- Small fiber neuropathy
- Fatigue
- Cognitive decline
- Pain Syndromes

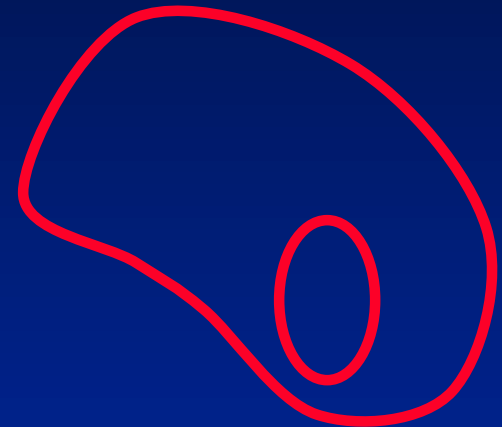
Sarcoidosis: Disordered Vitamin D Metabolism

Alveolar macrophage



25-OH vitamin D

1- α hydroxylase



1, 25-OH vitamin D

25-OH Vit D: **LOW**

1,25-diOH Vit D: **NL, HIGH**

PTH: **LOW**

Increased gut Ca⁺⁺ absorption

Hypercaluria (AMC: 174/269)

Hypercalcemia (AMC: 95/269)

nephrolithiasis

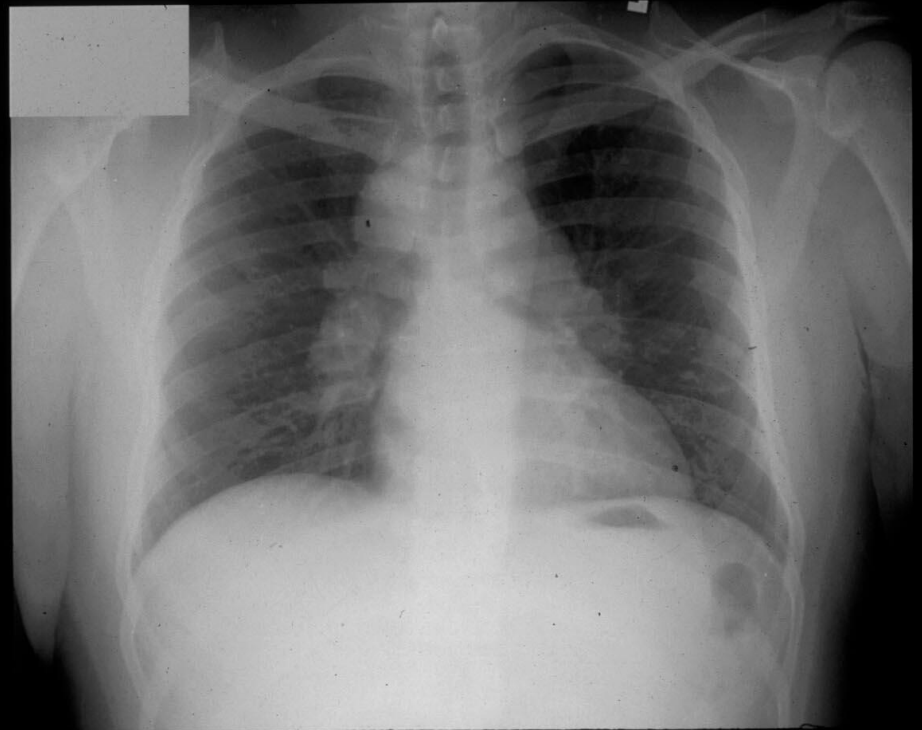
Lofgren's Syndrome

erythema nodosum

CXR: BHA

fever

ankle arthritis



Good prognosis

sarcoidosis small fiber neuropathy

- may occur in $\frac{1}{4}$ of sarcoidosis patients.¹
- unmyelinated c and thinly myelinated alpha-delta fibers²
- thermal/nociceptive: **painful neuropathy**²
 - burning, shocking
 - patchy
- autonomic system: **autonomic neuropathy**²
 - sweating
 - tachycardia
 - bladder/bowel

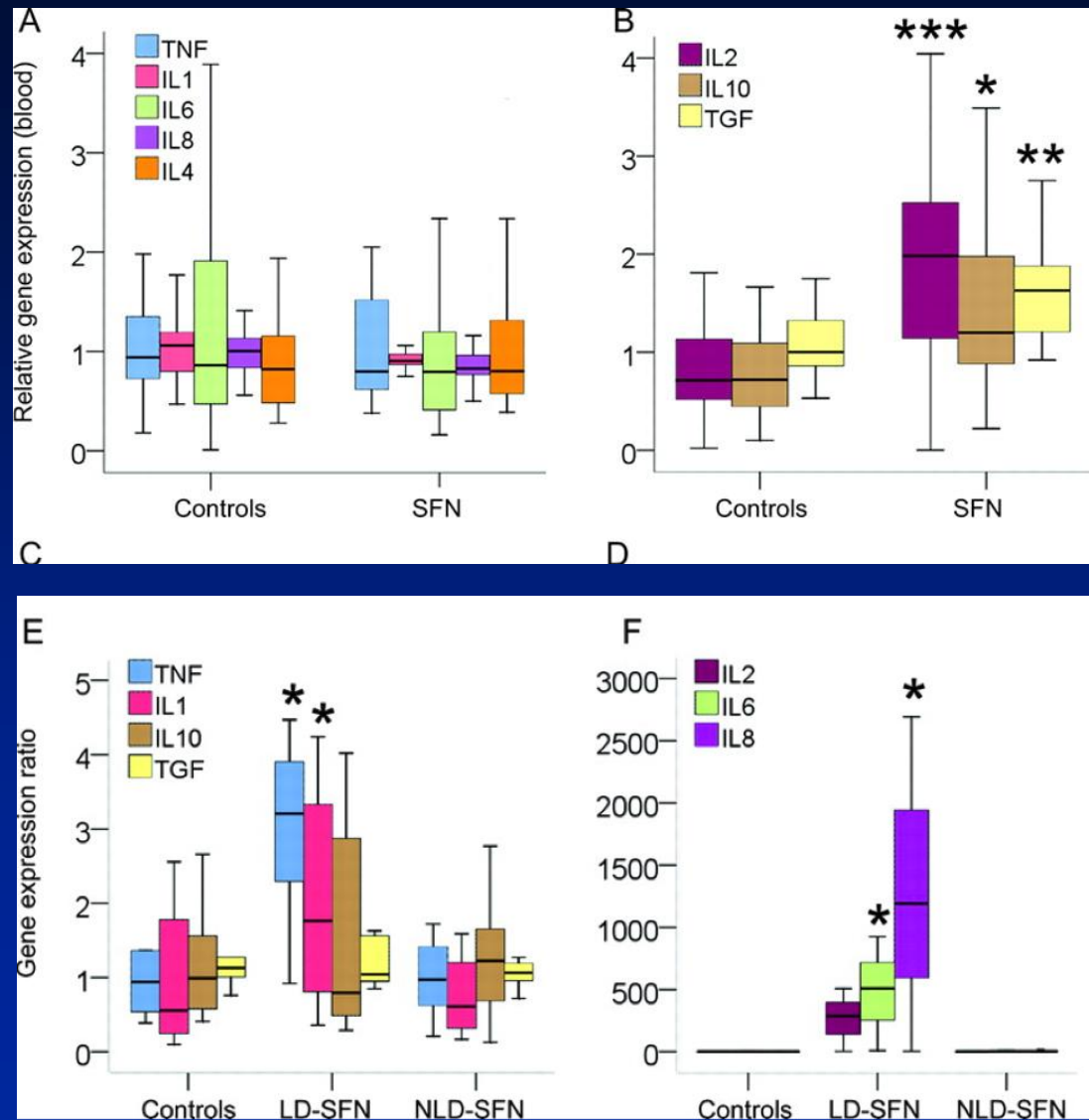
1. Tavee J. Cur Pain Headache Rep 2011; 15:201

2. Devigili G. Brain 2008; 131:1912

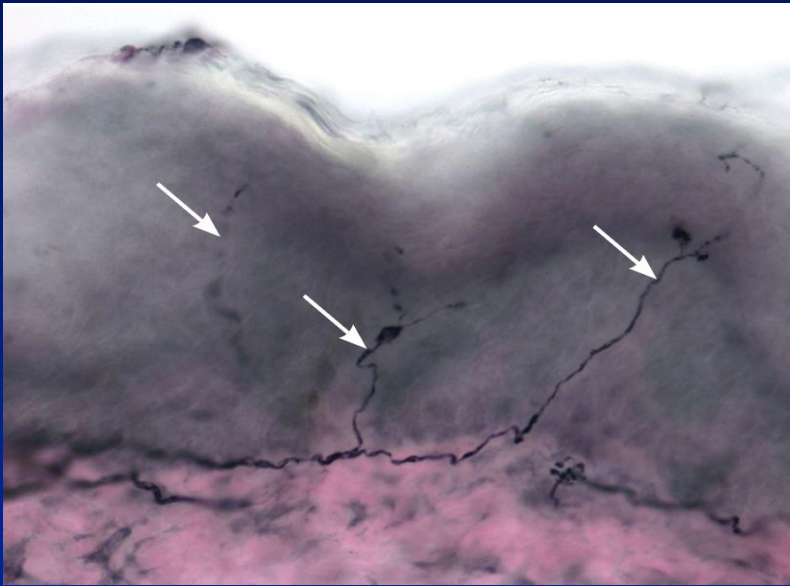
Sarcoidosis SFN: Pathophysiology

- Axon loss
- Cytokines and immune factors
 - Increased gene expression of local and systemic cytokines
 - Responsive to anti-TNF α agents
- Oxidative stress

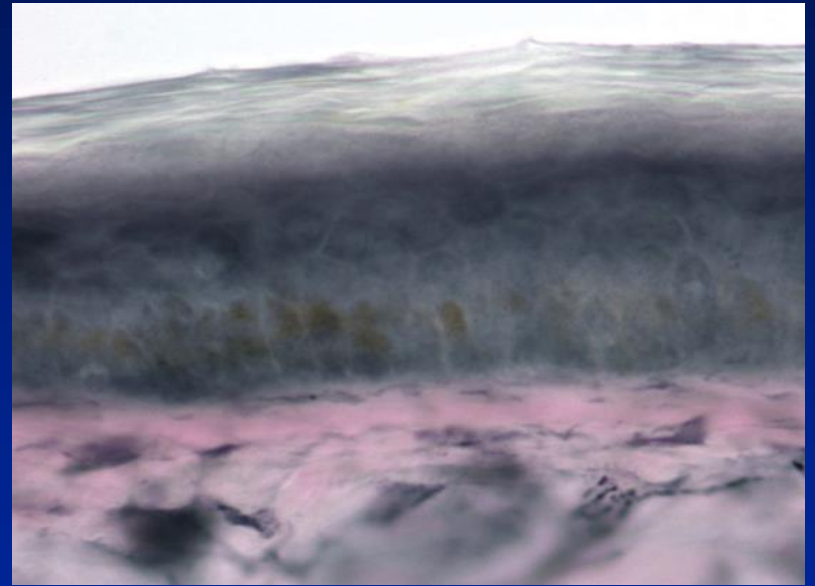
J. Tavee, Cleveland Clinic



Skin Biopsy for the Diagnosis of Small Fiber Neuropathy



Normal



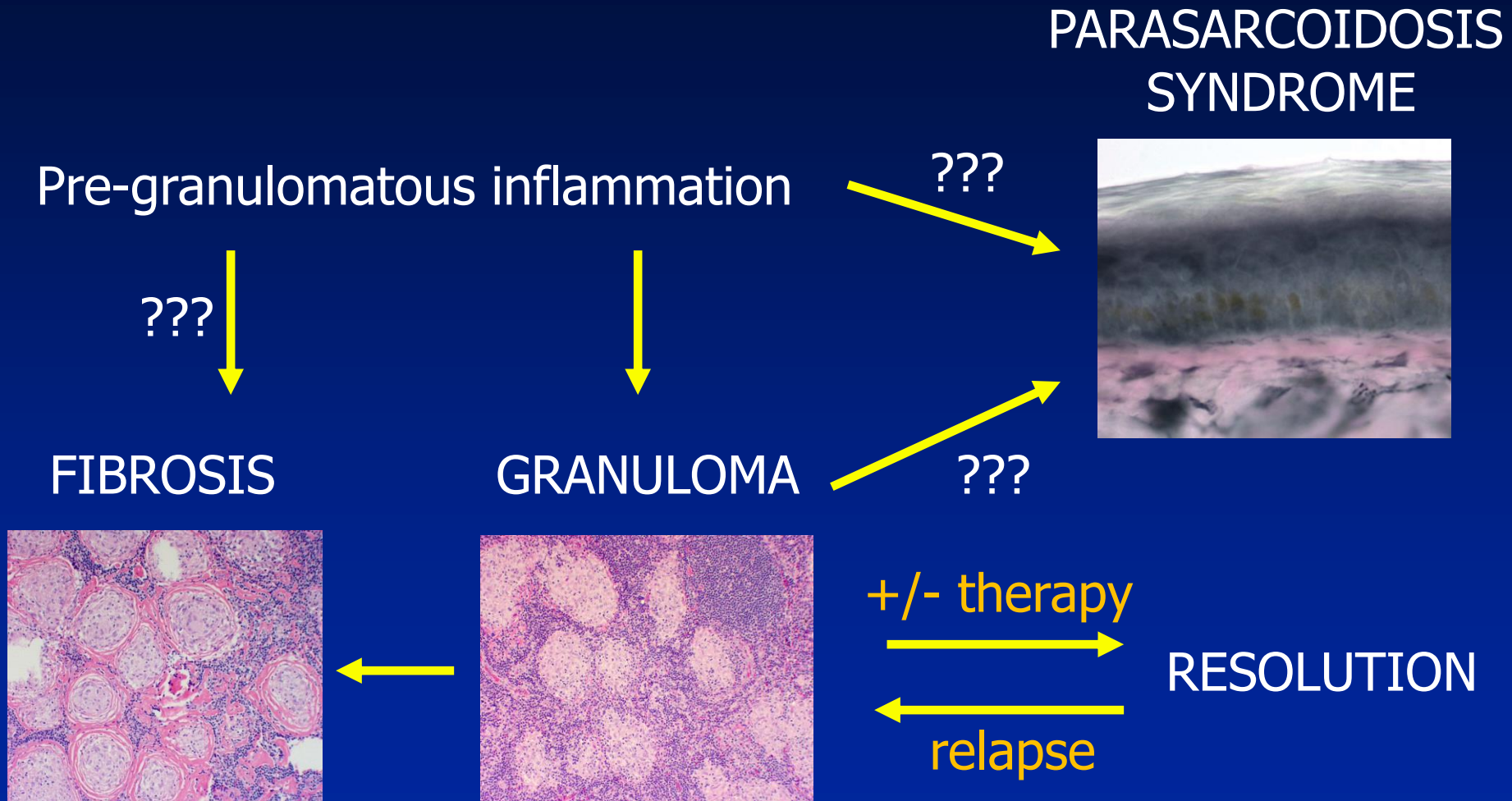
Small Fiber Neuropathy

Other Parasaroidosis syndromes

- **Fatigue (70%)¹**
- **Cognitive decline (35%)²**
- **Pain syndromes (70%)³**

1. De Kleijn WP. Sarcoidosis Vasc Diff Lung Dis. 2009; 26:92
2. Elfferich MD. Respiration. 2010; 80:212
3. Hoitsma E. Sarcoidosis Vasc Diff Lung Dis. 2003; 20:33

The origin of parasarcoidosis syndromes is unknown

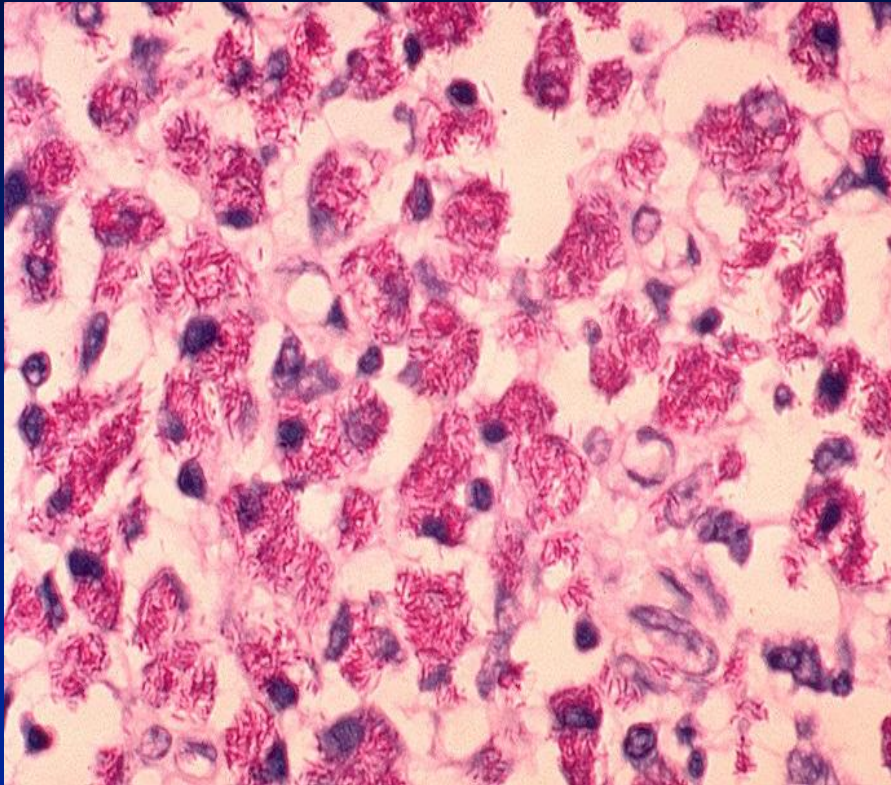


4: Antigen. Hypothesis: Sarcoidosis involves an interaction between the environment (exposure) and genetically susceptible individuals that causes granulomatous inflammation

- The exposure is currently unknown
- May be many exposures depending on the host (genetics)
- May be molecular mimicry: host response cross reacts with autoantigens
- May be immune dysregulation or a normal immune response

Potential Sarcoidosis Antigens:

Infectious: mycobacteria



Mycobacterial catalase-peroxidase (MKatG)

Present in sarcoid tissue.¹

Similar properties as Kveim agent.¹

T-cell response to MkatG in PBMC of sarcoidosis patients.²

T-cell response in to MKatG In BAL of Sarcoidosis patients.³

1. Song Z. J Exp Med 2005; 201:755

2. Chen ES. J Immunol 2008; 181:8784

3. Oswald-Richter KA. Infect Immunol 2009; 77:3740

Potential Sarcoidosis Antigens:

Infectious: *propionibacterium acne*



propionibacterium acne

Present in DNA of lymph
of Japanese and Europeans
With sarcoidosis.¹

1. Eishi Y. J Clin Microbiol 2002; 40:198-204

Sarcoidosis: infectious agents

- steroids improve sarcoidosis implies
 - sarcoidosis is not caused **directly** from a pathogen
 - Sarcoidosis may be caused from a **host response** to an antigen of an infectious agent
 - e.g. ABPA

Potential Sarcoidosis Antigens: Occupational/Environmental: Metals



Titanium

Kucera GP Chest 2003; 123:1527



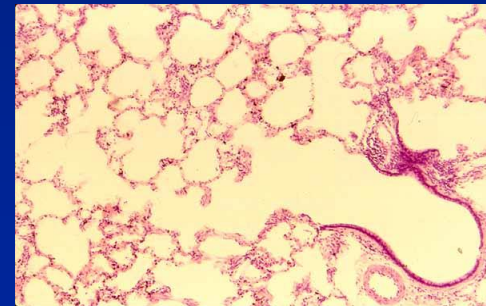
Photocopier toner (Si, Fe, Cu)

Rybicki BA SVDLD 2004; 21:49



Metal work

Kucera GP Chest 2003; 123:1527



Lung biopsy (Si, Al, Ti)

Drent M. Respir Med 2004; 94:815

Potential Sarcoidosis Antigens: Occupational/Environmental: Combustion products



World Trade CTR dust
Izbicki G. Chest 2007; 131:1414

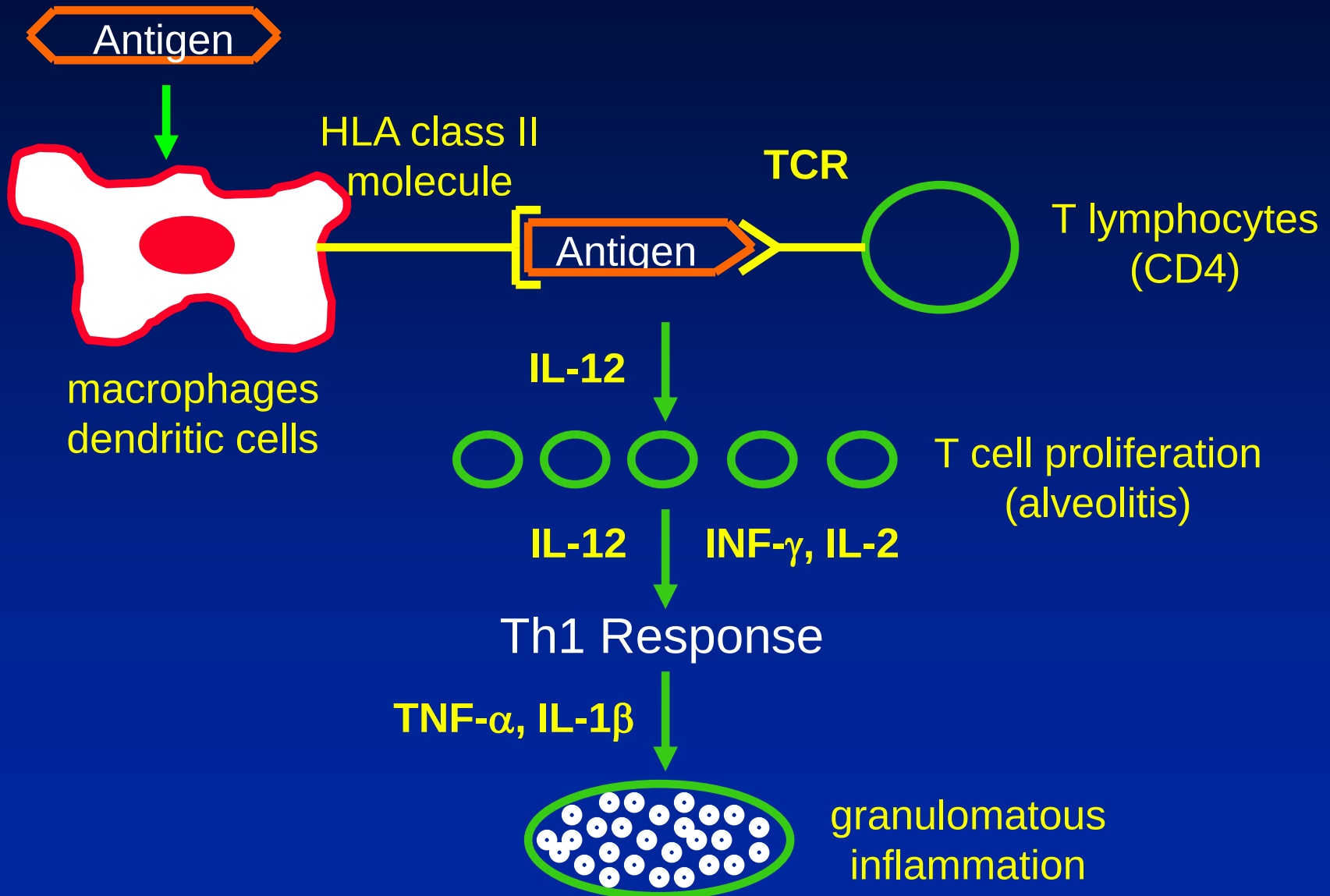


Firefighters
Prezant DJ. Chest 1999; 116:1183



Wood stove exposure
Kajdasz DK. Ann Epidemiol
1999; 150:271

Immunologic Basis of Sarcoidosis



T cell, Antigen, APC

T Cell

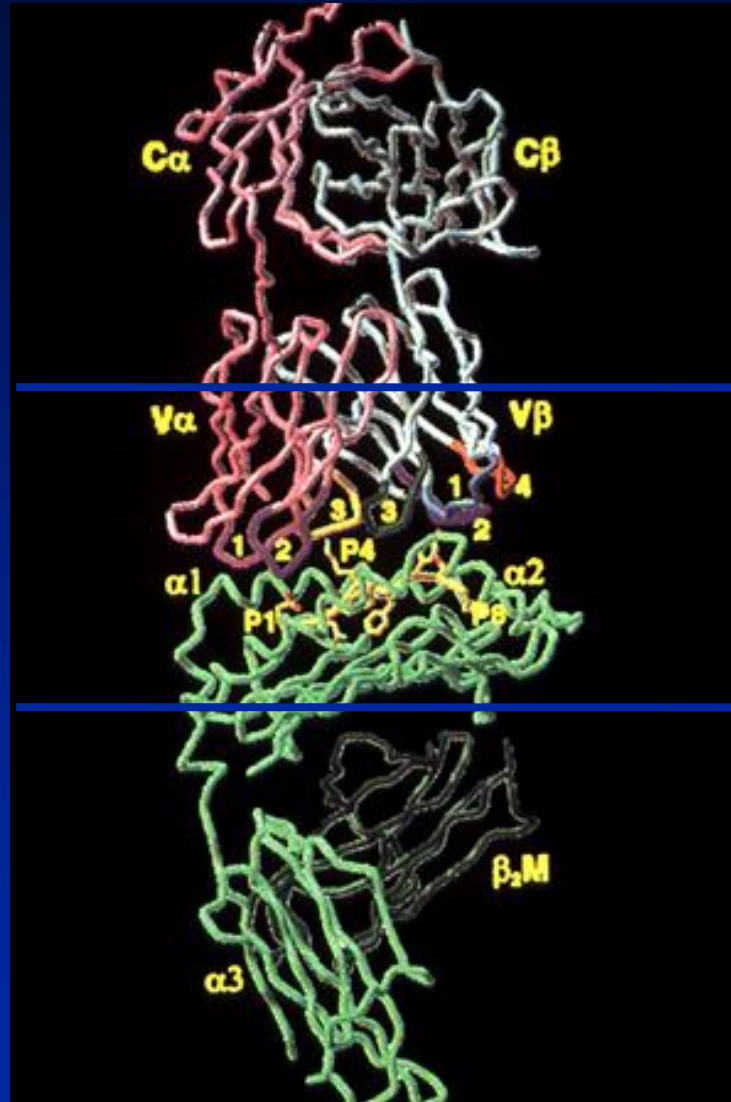
T Cell
Antigen receptor

Binding
site

APC

Antigen (yellow)

HLA



5: Genetics

- HLA
- Non-HLA

HLA and Sarcoidosis: Good Prognosis

- **DR3**
 - Italians, Czechs, English, Scandinavians
- **B8**
 - Italians, English
- **DR52**
 - Japanese
- **C4AQ0**
 - Italians

Sarc Vasc Diff Lung Dis 2002; 19:83

Sarcoidosis and HLA: Poor Prognosis

- DR5
 - Japanese, Italians
- DR8
 - Japanese
- B35
 - Italians
- DQ7
 - Italians

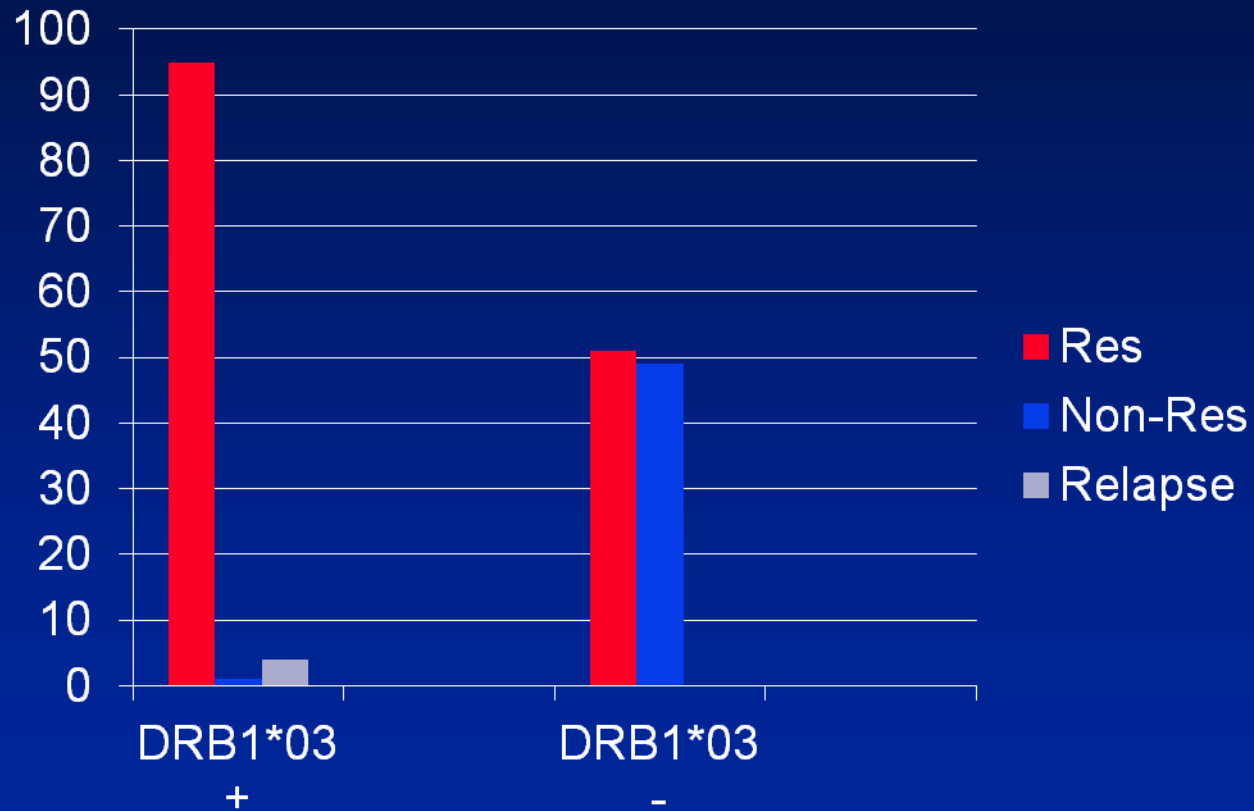
Sarc Vasc Diff Lung Dis 2002; 19:83

Sarcoidosis and HLA

- **acute form**
 - B8/DR3: English, DRB1*03: Polish, Scandinavians, DRB3*1010: Polish, TNFA*2: Germans
- **early onset**
 - DR3: Japanese, B35, DR5: Italians
- **cardiac sarcoidosis**
 - DQB1*0601: Japanese
- **eye sarcoidosis**
 - DR52: Japanese

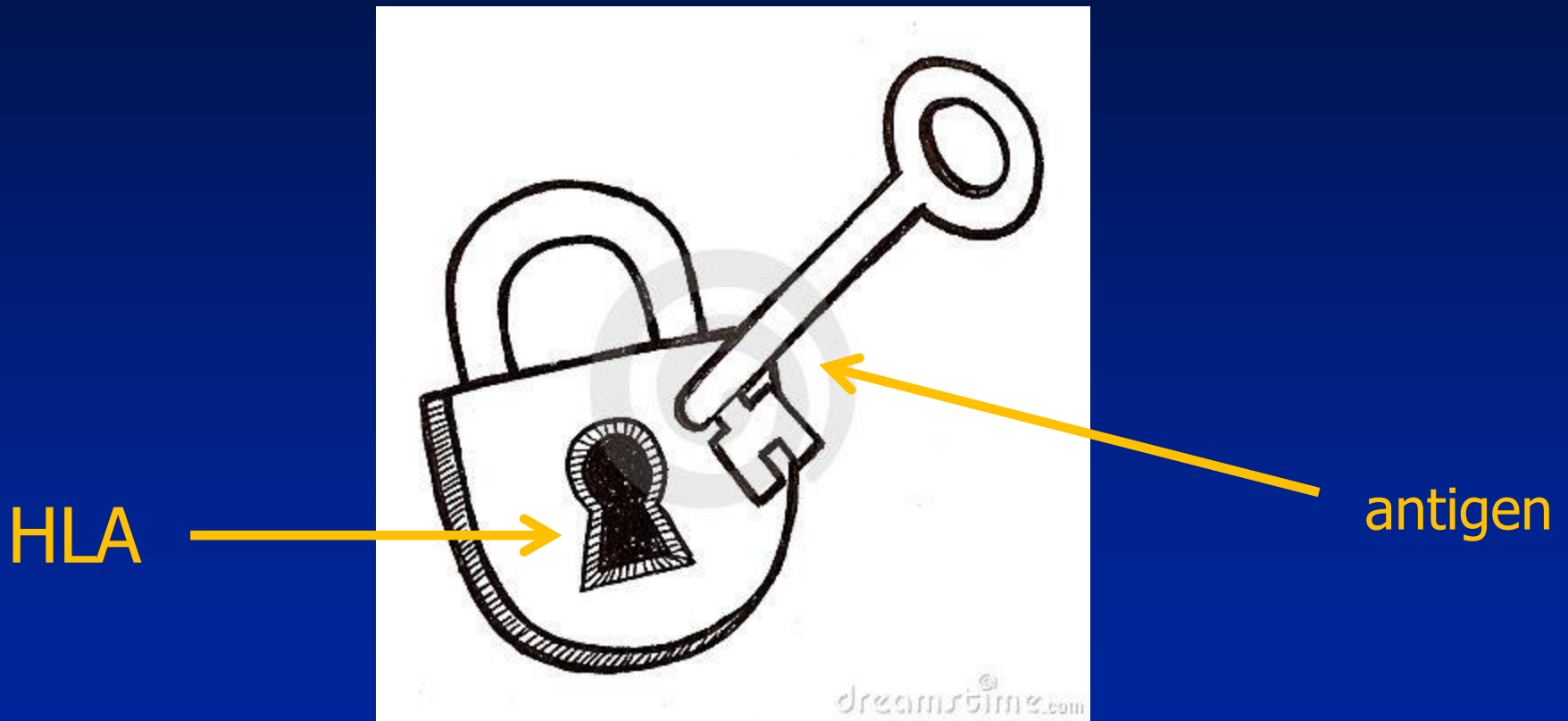
Sarc Vasc Diff Lung Dis 2002; 19:83

HLA: DRB1*03 in Sweden



Grunewald J. Am J Respir Crit Care Med 2009; 179:307

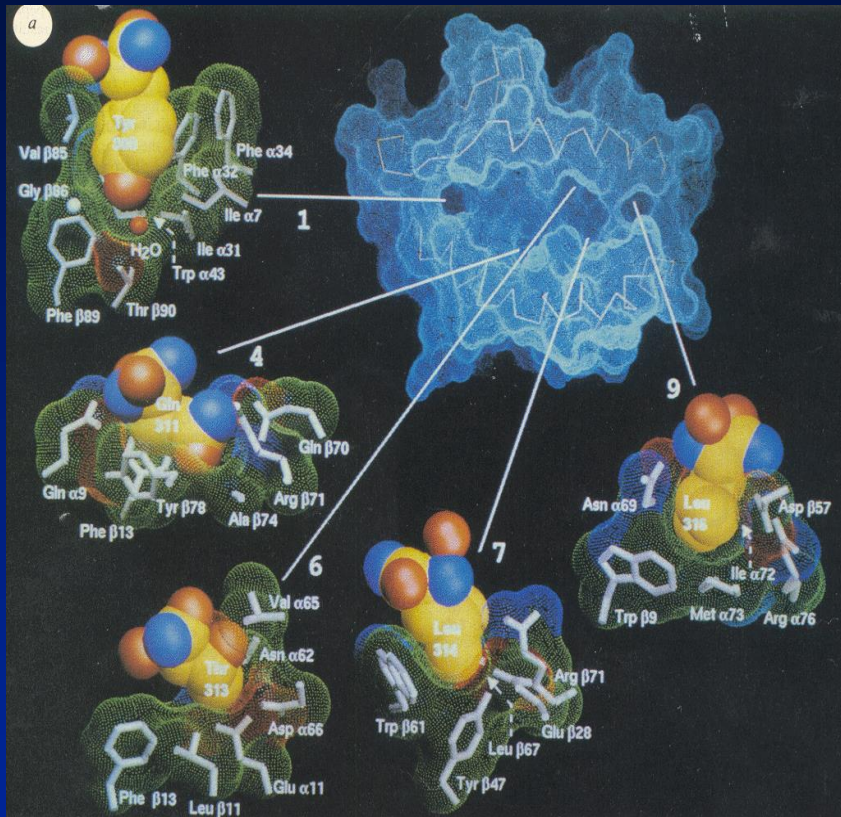
HLA and antigen: lock and key



Exposure-Genetics-Phenotype Hypothesis

exposure	genetics (? HLA, TCR)	sarcoidosis	phenotype
A	X	+	I
A	Y	-	
B	X	-	
B	Y	+	II

Binding Pockets in HLA



Associated with sarcoidosis:

USA: DRB1*1101
USA: DPB1*0101
UK: CW7,DPB1
Italy: C4AQ0, BfF
Japan: DRW52, DMB*0102

Am J Hum Genet 2003; 73:720
Sarc Vasc Diff Lung Dis 2002; 19:83

Nature
1994:369;220

Etiology of Sarcoidosis

Maybe there are multiple causes...

The Sarcoidoses

Other genetic studies

- Genome-wide association studies (GWAS)
 - Annexin11 – Europeans and African Americans¹
 - rs1040461 – German and African American²
 - NOTCH4 – African Americans²

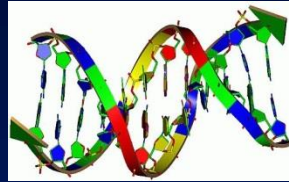
1. Hofmann S. Nat Genet 2008; 40:1103

2. Adrianto I. PloS One 2012; 7:e43907

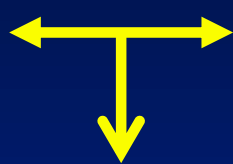
Sarcoidosis involves an exposure – genetic interaction



Exposure



Genetics

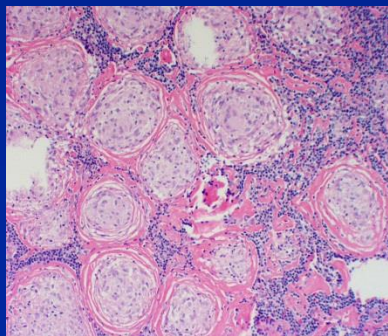


Pre-granulomatous inflammation

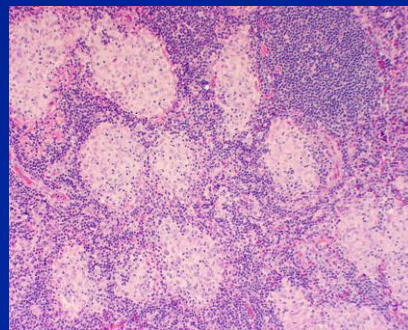
???



FIBROSIS

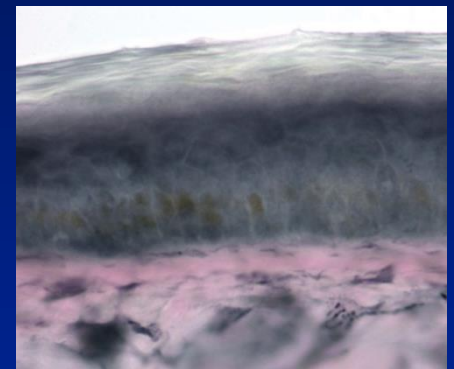


GRANULOMA



PARASARCOIDOSIS
SYNDROME

???



???



+/- therapy

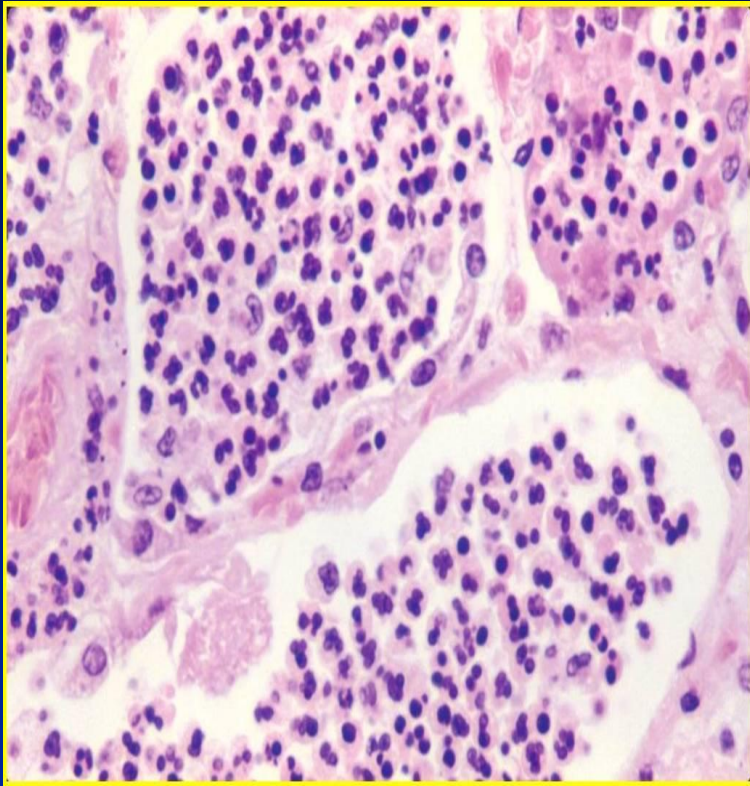


RESOLUTION

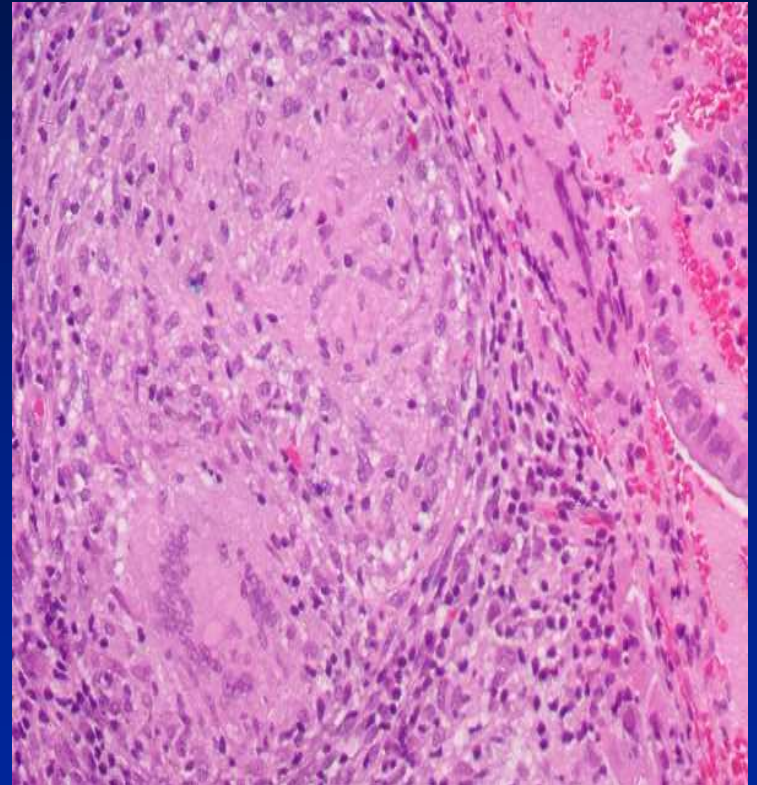


relapse

6: Pre-granulomatous inflammation: What makes a granuloma?



Pneumococcal
pneumonia



Tuberculosis

5. Pre-granulomatous inflammation: What makes a granuloma?

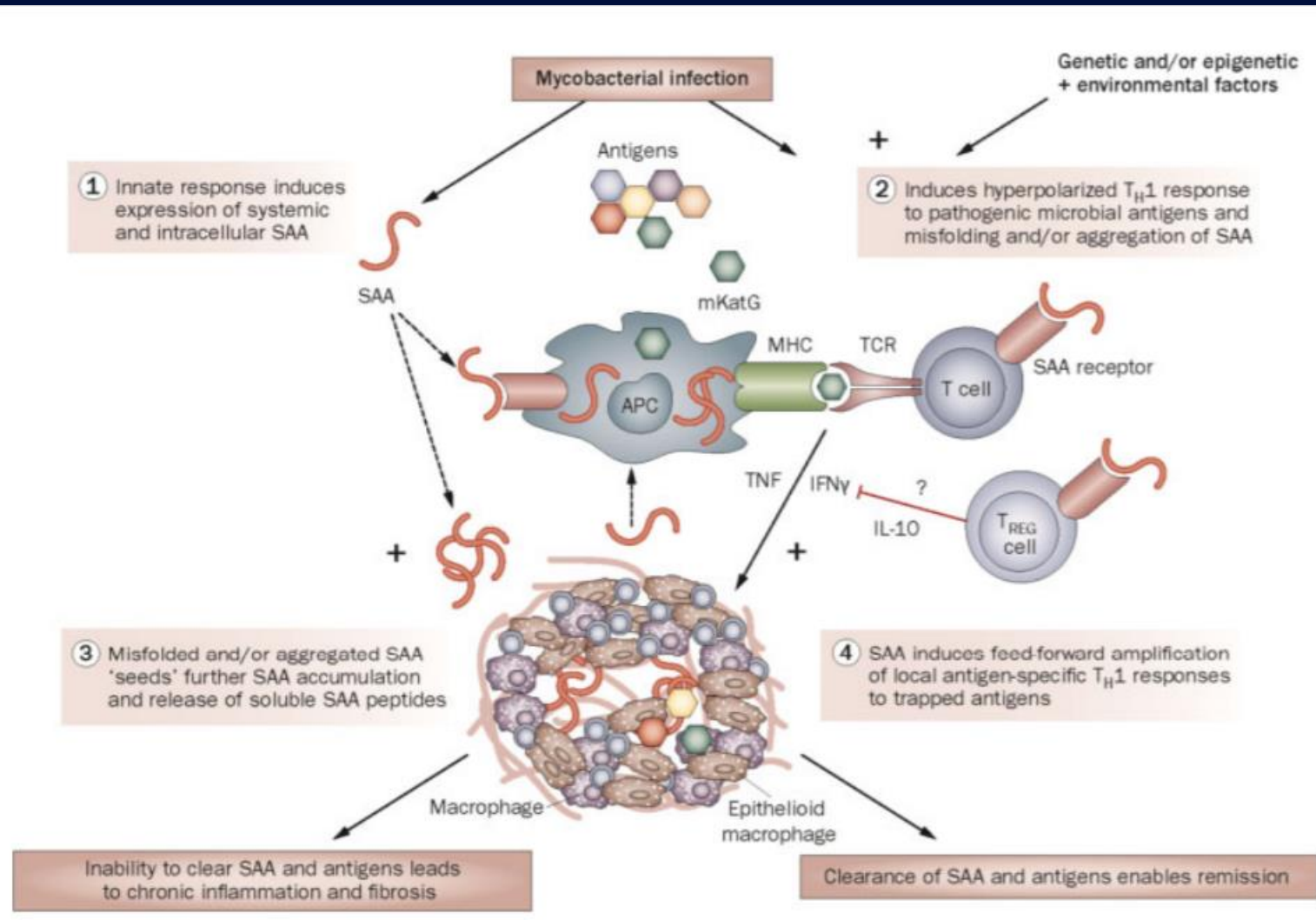
- Granulomas usually form as a result of a **non-degradable product** and/or the result of a delayed-type hypersensitivity (DTH) response.¹
- Immune system exhaustion?

1. Kobayashi K *Microsc. Res. Tech.* 2001; **53**: 241–5

Sarcoidosis and SAA

- serum amyloid A (SAA) is found within the sarcoid granuloma
- very specific for sarcoidosis
- originates from macrophages
- consolidate a poorly soluble protein aggregate
- may disrupt the clearance of an antigen within the granuloma that allows for its persistence
- **T-cell exhaustion**

SAA in sarcoidosis



Cells and cellular products involved in the formation of the sarcoid granuloma

- Granuloma induction
 - T-helper-1 cells (Th-1)
 - TNF- α
- CXCL9, CXCL10, CXCL 11
 - Released by macrophages and attract Th1 cells¹
- Regulatory T-cells (T-reg)
 - High numbers in Lofgren's syndrome²
 - Reduced in chronic disease³
 - Anergy?
- Th-17 cells
 - Produced in active sarcoidosis⁴
 - Some produce IL-17 AND INF: Th17.1 cells⁵

-
1. Morgan AJ. Clin Exp Allergy 2005;35:1572
 2. Miyara M. J Exp Med 2006;203:359
 3. Idall F. Clin Exp Immunol 2008;152:127
 4. Zissel G. Clin Chest Med 2015; 36:549
 5. Ramstein J. AJRCCM 2016; 193:1281

Cells and cellular products involved in the formation of the sarcoid granuloma

- TOR signaling pathway¹
- IL-13 signaling pathway²
- CD163, M2-related marker
 - In macrophages and giant cells of sarcoid granulomas³
- JAK pathway activation⁴
- Dendritic cells⁵

1. Calendar A. Eur Respir J 2019 (in press)
2. Crouser ED. Am J Respir Cell Mol Biol 2017; 57:487
3. Shamaei M. Human Immunol 2018; 79:63
4. Zhou T. Sci Rep 2017; 7:4237
5. Ten BB. Respir Res 2012

? Major role of dendritic cells in sarcoidosis?

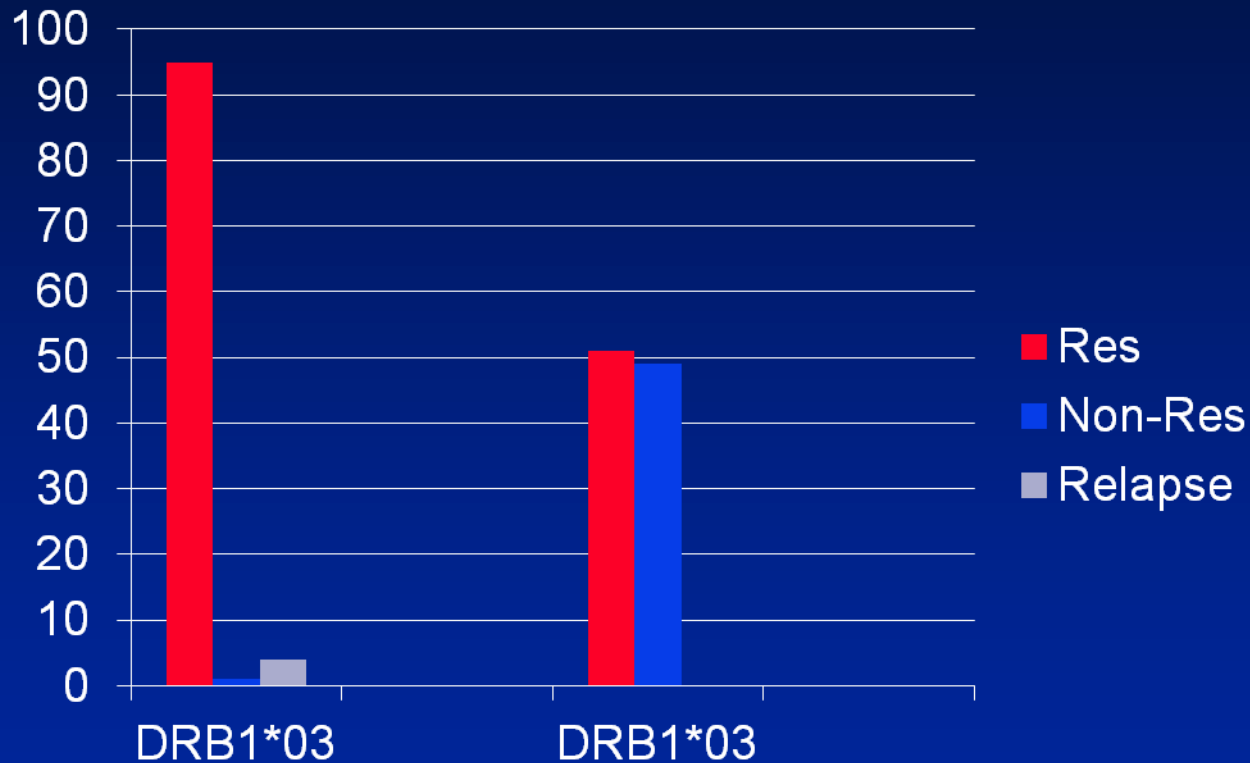
TABLE 2] Most Common Patterns of Organ Involvement in NPS

Organ Involvement	No. (%)
Isolated skin	35 (25.0)
Skin + eye	7 (5.0)
Extrathoracic lymph node	6 (4.3)
Isolated eye	5 (3.6)
Isolated ENT	5 (3.6)
Isolated neurologic	5 (3.6)
Skin + liver	3 (2.1)
Skin + bone	3 (2.1)
Skin + eye + liver	3 (2.1)
Liver + spleen + bone marrow	3 (2.1)
Liver only	3 (2.1)
Cardiac only	3 (2.1)
Other	57 (40.7)

ENT = ear, nose, and throat; NPS = nonpulmonary sarcoidosis.

7. Molecular mimicry? HLA: DRB1*03

- Vimentin has been identified as an autoantigen in these patients¹
- Patients developed anti-vimentin Abs and functional T-cell responses to vimentin²



Grunewald J. Am J Respir Crit Care Med 2009; 179:307

1. Kinlock AJ. Funt Immunol 2018; July 9
2. Musaelyan A. Autoimmun Rev 2018; 17:926

? Major role of dendritic cells in sarcoidosis?

TABLE 2] Most Common Patterns of Organ Involvement in NPS

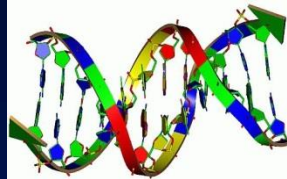
Organ Involvement	No. (%)
Isolated skin	35 (25.0)
Skin + eye	7 (5.0)
Extrathoracic lymph node	6 (4.3)
Isolated eye	5 (3.6)
Isolated ENT	5 (3.6)
Isolated neurologic	5 (3.6)
Skin + liver	3 (2.1)
Skin + bone	3 (2.1)
Skin + eye + liver	3 (2.1)
Liver + spleen + bone marrow	3 (2.1)
Liver only	3 (2.1)
Cardiac only	3 (2.1)
Other	57 (40.7)

ENT = ear, nose, and throat; NPS = nonpulmonary sarcoidosis.

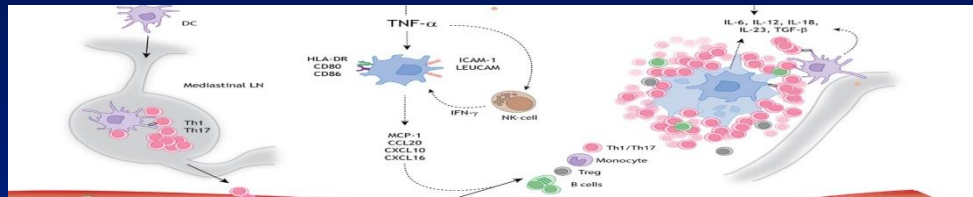
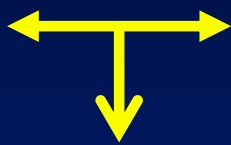
Immunopathology of sarcoidosis



EXPOSURE

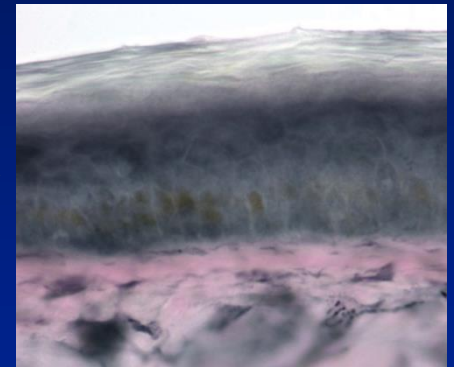


GENETICS



PRE-GRANULOMATOUS INFLAMMATION

PARASARCOIDOSIS SYNDROME



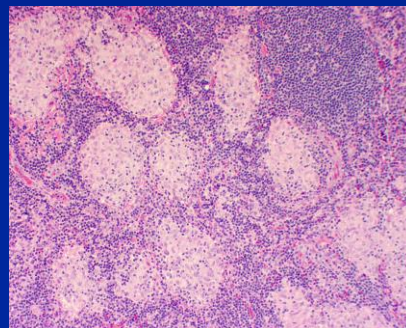
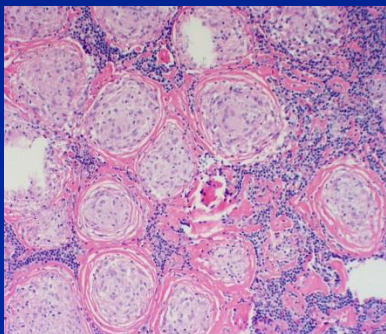
FIBROSIS

MOLECULAR MIMICRY

GRANULOMA

???

???



+/- therapy

RESOLUTION

relapse

