In the name of God
Pathogenesis of Sarcoidosis

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Sarcoidosis

- Multisystem inflammatory disease
- Unknown cause
- Noncaseating granulomata in any organ system
- Sarcoidosis occurs worldwide
- Affects people of all racial and ethnic backgrounds

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Inflammatory diseases
(General considerations)

- Triggering factors
- Genetic susceptibility
- Altering immune system
- Inflammatory response
POSSIBLE ETIOLOGIES

• The multicenter NIH-funded ACCESS case-control study of over 700 patients and nearly 30,000 relatives has been completed.

• No single etiologic agent or genetic locus was clearly implicated in the pathogenesis of sarcoidosis.

Etiology

• No definite etiologic agent
• Two major categories of agents
  • Microbial organisms
  • Noninfectious environmental agents

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Microbial Agents

- The longest and widely studied agent is mycobacteria.

- A *meta-analysis of several studies* showed that over 26% of sarcoidosis biopsy specimens had evidence of mycobacterial DNA.

Potential role of mycobacteria

• The routine histological analysis of pathologic specimens does not reveal microbial organisms.

• There are no reproduced studies that document the presence of viable mycobacteria.

The American Journal of Medicine (2012) 125, 118-125
Potential role of mycobacteria

• The mycobacterial catalase-peroxidase (mKatG) protein as a candidate pathogenic antigen.

• The mKatG protein is a virulence factor for *Mycobacterium tuberculosis*.

Nat Rev Rheumatol 2011; 7:457
**Microbial Agents**

- mKatG *in most sarcoidosis biopsy specimens*, but in none of the disease-free controls.
  

- Circulating IgG directed to mKatG protein in approximately 50% of sarcoidosis patients.
  

- An increased BAL T-cell reactivity to *mKatG has recently* been shown in sarcoidosis patients.
  
  *The American Journal of Medicine (2012) 125, 118-125*
Role of mycobacteria

- Genomic or protein material of mycobacterial origin in sarcoidosis tissues.
- Elevated humoral and cellular immune response to mycobacterial antigens.
- Support the hypothesis that mycobacterial antigens may drive some cases of sarcoidosis.

Role of mycobacteria

• No evidence of an active infection or reactivation of a latent TB in sarcoidosis.

• Many of them undergo years of corticosteroid, immunosuppressive or anti-TNF therapy.

Nat Rev Rheumatol 2011; 7:457
Potential role of propionibacteria

• A Japanese study found DNA from *Propionibacterium* spp. in almost all tissue samples from patients with sarcoidosis.

• But also in most of the tissue samples from healthy controls.

• *T-cell proliferation in response to P. acnes proteins was greater in patients with sarcoidosis than in those without sarcoidosis.*

Nat Rev Rheumatol 2011; 7:457
Microbial Agents

- Other microbial agents, such as viruses (EB virus, herpes simplex virus, human herpes virus-8), fungi, spirochetes, Borrelia species, and *Tropheryma whipplei*, have been suggested as possible etiologic agents of sarcoidosis.

- Most of the studies lack strong evidence to support any of the suggested microbes.

Microbial Agents? (transplantation)

- The occurrence of sarcoidosis in individuals receiving bone marrow transplantation from sarcoidosis patients.

- Occurrence of sarcoidosis in lung and heart allografts following transplantation are suggestive of a transmittable agent.

- Antigenic agents in the mononuclear phagocytes of sarcoidosis patients.

Environmental risks reported include:

- rural living
- exposure to pine pollen
- lumber industry
- pica, fireplaces
- wood stoves
- clay eating
- inhalation of peanut dust
- hair spray
- home mold exposure

Environmental Agents
( Occupational risks for sarcoidosis )

• Military personnel, fire rescue workers, industries where pesticides are used, and health care personnel.

• Many of the studies did not include well-matched control groups.

• An incidence of 86 per 100,000 during the year following World Trade Center incident in 2001 in New York City compared with 22 per 100,000 cases during the prior 4 years.

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Environmental Agents
(ACCESS study)

Environmental exposures with an odds ratio risk of 1.5:
- Agriculture-related occupations
- Mold or musty odors at work
- Exposure to pesticides

One strong negative association was noted in smokers.

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Environmental Agents
(Granulomatous inflammation)

- Exposure to inorganic agents, such as beryllium, zirconium, nickel, chromium, and synthetic mineral fibers.

- Chronic beryllium lung disease findings resemble that of sarcoidosis.

- However, chronic beryllium disease does not have the typical extrathoracic findings of sarcoidosis.

Genetic factors

• The findings of multiple studies indicate that genetic factors have a role in determining the risk and clinical course of sarcoidosis.


• A positive family history of sarcoidosis was noted in 17% of black Americans but only in 1.4% of Spanish individuals with this disorder.

  *Arch. Bronconeumol. 43, 92–100 (2007)*
Genetic factors

• The ACCESS study found a fivefold increased risk among siblings and all first-degree relatives of affected individuals.  

A Scandinavian registry study found that compared with the general population:

• Co-twins of monozygotic siblings had an 80-fold increased risk, whereas risk in dizygotic twins was only sevenfold.  
  *Thorax 63, 894–896 (2008).*
HLA genes in sarcoidosis

• Both HLA class I and class II genes have been associated with the risk of sarcoidosis.

• In the ACCESS study, the HLA-DRB*1101, HLA-DRB*1201, HLA-DRB*1501 and HLA-DRB*0402 alleles had the strongest association with the risk of sarcoidosis.

Nat Rev Rheumatol 2011; 7:457
HLA genes in sarcoidosis

- HLA-DRB1*0301 and HLA-DQB1*0201 are strongly associated with remitting sarcoidosis.

- whereas the haplotype HLA-DQB1*0602-DRB1*150101 is associated with chronic, unremitting disease.

Nat Rev Rheumatol 2011; 7:457
Non-HLA genes in sarcoidosis

- TNF is a critical mediator of granulomatous inflammation.

- Variants in the TNF gene have been associated with a 1.5-fold increased risk of developing sarcoidosis.

*J. Hum. Genet. 52, 836–847 (2007).*
Non-HLA genes in sarcoidosis

• Variants in the gene encoding the transmembrane receptor RAGE have also been associated with increased risk of developing sarcoidosis.

• Since this gene is located within the MHC region it remains unclear whether the association is attributable to linkage with nearby HLA genes.

Nat Rev Rheumatol 2011; 7:457
Findings of genome-wide association studies (BTNL2 gene)

- Polymorphisms in the butyrophilin-like 2 (BTNL2) gene that are associated with increased risk of sarcoidosis.

- BTNL2 protein is structurally related to the B7 family of costimulatory molecules.

- Since BTNL2 is located within the MHC locus, it is possible that linkage to other MHC genes could explain the association with sarcoidosis.

Findings of genome-wide association studies

- Other candidate susceptibility genes include ANXA11.
- Which encodes a molecule involved in apoptosis and cellular proliferation.
- FAM178A, a gene of unknown function that is associated with Crohn disease.
- The mechanistic pathways relevant to the pathogenesis of sarcoidosis remain to be defined.

Nat Rev Rheumatol 2011; 7:457
Immunopathogenesis of Sarcoidosis
ANTIGEN PROCESSING

Antigen presenting cell
ANTIGEN PROCESSING
ANTIGEN PROCESSING
ANTIGEN PROCESSING

DR

IL-1
ANTIGEN RECOGNITION

ANTIGEN

APC

DR

T-CELL
ANTIGEN RECOGNITION

ANTIGEN

DR

CD4+

APC

T-CELL
ANTIGEN RECOGNITION

ANTIGEN

DR

APC

CD4+

CD80/86

CD28

T-CELL
ANTIGEN RECOGNITION

ANTIGEN

DR

APC

CD80/86

CD28

CD4+

Cytokines

T-CELL

Cytokines
**Resting APC**
- MHC
- CD28

**Naive T cell**
- T-cell receptor

**No response**

**Activated APC**
- MHC
- T-cell receptor
- B7
- CD28

**T cell**
- Interleukin-2

**T-cell proliferation, differentiation, survival**

**Activated APC**
- MHC
- T-cell receptor
- B7
- CD28

**T cell**
- Interleukin-2

**Down-regulation, functional inactivation**
The Control of Activated CD4+ T Cells by Regulatory T cells

NKT cells/CD4+CD25+ cells
CD4+CD25- cells

peptide/APC

Resting CD4 T cells
Activated CD4 T cells

(-)

Apoptosis

IL-12/IFN-γ
IL-4

(-)

TH1 CD4+ cells
TH2 CD4+ cells

IFN-γ
IL-10

(-)

CD8 or CD4 suppressor effector
CD8 or CD4 suppressor precursor

Regulatory immunity
CD4/CD8 interactions
Th1 and Th2 CD4+ T Cells

**Th1**
- IL-12 induces differentiation
- Cytokine Production:
  - Interferon-γ
  - Interleukin-2
- Intracellular Pathogens
- Macrophage Activation
- Delayed Type Hypersensitivity

**Th2**
- IL-4 induces differentiation
- Cytokine Production:
  - Interleukin-4
  - Interleukin-5
  - Interleukin-10
  - Interleukin-13
- Extracellular Pathogens
- B Cell activation & IgE
- Eosinophil responses
- Immediate Type Hypersensitivity
INITIATION PHASE: Antigen processing and presentation and T-cell activation

Susceptibility risk factors
eg, HLA risk alleles, BTN2L2, Annexin A11

Unidentified antigens (eg, mKatG)

TLR2

Serum amyloid A

APC

MHC II

MHC II

Activation

Naïve CD4 T cell

Activated CD4 T cell

Local oligoclonal proliferation

Proinflammatory response

CD4 T cell

Macrophage

TNF

CXCL10

CCR2

CCL2

CCL3

CCL4

CCR5

JAMA, January 26, 2011—Vol 305, No. 4
Immunology of sarcoidosis

- The proportion of T cells is increased in bronchoalveolar fluid from patients with sarcoidosis.
- CD4+ T cells dominate, with a CD4+:CD8+ T-cell ratio typically >3–5:1 compared with a ratio of 2:1 in healthy persons.

TH1 polarization

- Highly polarized expression of cytokines associated with a TH1 response.
- The expression of IFN-γ, the signature cytokine produced by TH1 cells.
- IL-12, IL-18 and IL-27, cytokines that promote a TH1 response, is upregulated.
- IL-2 and IL-15 are upregulated in patients with sarcoidosis, and have a proliferative and antiapoptotic effect on T cells.

Nat Rev Rheumatol 2011; 7:457
TH1 polarization

• Chemokines and chemokine receptors typically associated with a TH1 response are also upregulated in sarcoidosis.

• By contrast, IL-4 and IL-5, cytokines released by TH2 cells are downregulated at sites of inflammation in patients with sarcoidosis.

*Thorax 66, 144–150 (2011)*
Role of TNF

- TNF is a known mediator of granuloma formation.
- TNF-inhibitor therapy improves lung function and extrapulmonary manifestations in individuals with sarcoidosis.

Regulatory T-lymphocytes.
Sarcoidosis is associated with a deficiency of naturally occurring TREG responses.

Whether this deficiency has a critical role in the pathogenesis of disease remains uncertain.

*Am. J. Pathol. 174, 497–508 (2009).*
Immunoregulatory cells

- Inhalation of vasoactive intestinal peptide (VIP), reduced the production of TNF by lung cells in patients with sarcoidosis.

- This reduction was associated with an increased frequency of CD4+CD127–CD25+ TREG cells in the lung.

- Leading the authors to suggest inhaled VIP as a treatment for exaggerated immune-mediated lung diseases, including sarcoidosis.

Mechanisms of fibrosis

- The mechanisms involved in a fibrotic outcome are unclear.

- IFN-γ downregulates fibroblast proliferation and extracellular matrix deposition.

- TGF-β, IGF-1 and matrix metalloproteinases can support a fibrotic response.

Nat Rev Rheumatol 2011; 7:457
Mechanisms of fibrosis

• In patients with fibrotic pulmonary sarcoidosis, alveolar macrophages express an alternative M2 phenotype.

• A shift from cytokines produced by Th1 cells to Th2 cells.

Serum Amyloid A Regulates Granulomatous Inflammation in Sarcoidosis through Toll-like Receptor-2

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Serum amyloid A

- Serum amyloid A (SAA) is an acute phase protein released by the liver.
- Its production is stimulated by IL1 and IL6.
- Serum amyloid A is an acute phase reactant.

Respiratory Medicine (2011) 105, 775e780
Serum amyloid A (SAA)

- SAA plays a variety of immunoinflammatory functions.
- It induces chemotaxis and adhesion molecule expression.
- It up-regulates metalloproteinasases.

Respiratory Medicine (2011) 105, 775e780
Serum amyloid A (SAA)

• There is great interest in SAA in sarcoidosis.

• Protein was recently proposed as a key regulator of granulomatous inflammation in this disease.

• Regulates Th-1 immune responses through interaction with Toll-like receptor.

Respiratory Medicine (2011) 105, 775e780
SAA misfolding hypothesis

- SAA may be up-regulated locally within the macrophage.

- Up-regulation of hepatic release of SAA induced as part of a systemic acute phase response.

- Third, IFN-g directly impairs the degradation of SAA.

- SAA binds to several matrix proteins such as laminin and vitronectin.

- Poorly soluble protein aggregate to form a nidus for granuloma formation.

Sarcoidosis Antigen is Discovered in Tissue Extracts

- A prior eradicated infection leaves behind protein antigens (debris)
  - A non-infectious, microbial trigger for sarcoidosis

- mKatG is likely one of many antigens
  - Other mycobacterial proteins?
    - Drake (Vanderbilt), Dubaniewicz (Poland)
  - Other microbes? propionibacteria (Japan)

- Additional questions beyond mKatG:
  - What causes granulomas and scarring?
  - What else can we learn from Kveim reagent?
Role of SAA in Sarcoidosis?

- Serum amyloid A is an acute phase reactant
- SAA can act as a chemical signal through several receptors, including TLR2, RAGE, and others
- SAA binds matrix proteins and may serve as an important granuloma structural component
  - May contribute to how granulomas may function as an “antigen depot” for circulating proteins and microbes

SAA in sarcoidosis

1. SAA is locally induced within APCs and up-regulated in a systemic APR.

2. Leaves remnants of mycobacterial protein antigens such as mKatG.

3. SAA serves a nidus for epithelioid granuloma formation.

SAA in sarcoidosis

4-SAA as a trap for microbial or autoantigens while soluble SAA, released from tissue granulomas, serves as a ligand for TLR2.

5-Granulomatous inflammation resolves only after concomitant clearance of SAA and local pathogenic antigens.

6-In unremitting sarcoidosis, ineffective degradation and clearance of SAA and pathogenic antigens.

Possible New Approaches to the Treatment of Sarcoidosis

- Reduce cytokine production by blocking binding of SAA to receptors
  - Identification of other SAA receptors
  - Reduce production of SAA
- Block important chemical signals that direct and fuel the inflammatory process
- Change or “re-educate” the immune system response to sarcoidosis antigens

Edward S. Chen, M.D. Johns Hopkins University Baltimore, MD USA 2012
conclusion

• Sarcoidosis is a multisystem disease.

• Characterized by noncaseating granulomatous inflammation.

• Sarcoidosis is thought to result from both genetic susceptibility and specific environmental triggers.

• Highly polarized T-helper1 cell immune response is a hallmark of sarcoidosis.
Thank you for your attention