Infectious Etiology of Sarcoidosis

Granuloma

"Given that the epithelioid granuloma is the pathological hallmark of sarcoidosis, any etiological agent must be capable of inducing this pattern of inflammation."

Granuloma

"The formation of granulomas, an ancient and preserved pathological response to (usually) foreign material, occurs around a nidos of poorly soluble or insoluble material that cannot be simply removed by a single cell. It is an organized structure that develops in an orchestrated manner over days to weeks."

Pathophysiology
Lymphocyte-Macrophage Interaction

Antigen Presentation

What is the antigen?

Cytokine Profiles

Microorganisms in sarcoidosis

Schaumann’s Body

In 1917 Schaumann postulated that the laminated, calcific inclusions in sarcoid granulomas represented remnants of transformed tubercle bacilli.
Mycobacteria

Molecular evidence for the role of mycobacteria in sarcoidosis: a meta-analysis
D. Gupta, R. Agarwal, A.N. Aggarwal and S.K. Jindal
Eur Respir J 2007; 30: 508–516

231/874 (26%) sarcoidosis patients in 31 studies had mycobacterial nucleic acids detected in lymph nodes

Mycobacterial Proteins in Sarcoid Tissue

Mycobacterial catalase–peroxidase is a tissue antigen and target of the adaptive immune response in systemic sarcoidosis

Moller’s Hypothesis

- Sarcoïdosis is caused by linked T- and B-cell response to poorly soluble protein aggregates of microbial and/or endogenous origins with physiochemical properties similar to those of the Kveim reagent.
- No a priori hypothesis regarding specific microbial, environmental, or auto-antigens

Moller’s approach

1. Limit the proteome set of tissue proteins on the basis of the physiochemical properties of the Kveim reagent

Kveim-Siltzbach Reaction

Kveim-Siltzbach Reaction:
Well-formed epithelioid granulomas develop 2-4 weeks following intradermal injection
Biochemical characterization of Kveim extracts revealed poorly soluble protein aggregates:
- insoluble in neutral detergents
- heat, acid, and protease resistant
- sensitive to potent denaturants
**Moller’s approach**

1. Limit the proteome set of tissue proteins on the basis of the physiochemical properties of the Kveim reagent
2. Assess for the presence of tissue antigens by protein immunoblotting, using sarcoidosis and control sera
3. Identify candidate antigens by matrix-associated laser desorption/ionization-time of flight mass spectrometry
4. Confirm the presence of candidate antigens in tissues by protein immunoblotting, using specific antibody reagents to the candidate protein
5. Evaluate B- and T-cell responses to recombinant candidate proteins and derived peptides identified by the initial studies

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**Protease-resistant proteins in sarcoidosis tissue**

Neutral detergent +/- protease

Song et al, JEM 2005;201:755-67

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**Mass Spectrometric analysis identifies mycobacterial proteins in protease resistant extracts**

- Mtb catalase-peroxidase (mKatG)
- Mtb topoisomerase 1

Song et al, JEM 2005;201:755-67

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**Anti-mKatG antibodies react with proteins in sarcoid tissue**

Song et al, JEM 2005;201:755-67

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**Sarcoid sera contains IgG that react with mKatG**

Immunohistochemistry identifies mKatG DNA sequences in 7/18 sarcoid tissue samples vs. 0/18 controls vs. 4/4 Mtb tissues

Song et al, JEM 2005;201:755-67
Summary

- mKatG identified by mass spectroscopy in protein extracts from sarcoid tissue
- Protein immunoblotting with anti-mKatG monoclonal antibodies confirmed the presence of mKatG in 5/9 sarcoid tissues vs. 0/11 control tissues
- IgG antibodies to mKatG were detected in the sera of 12/25 sarcoid patients vs. 0/11 control subjects

Song et al, JEM 2005;201:755-67

Monocytes from sarcoidosis patients secrete cytokines in response to Mycobacterial antigens

Multiple Mycobacterium antigens induce interferon-γ production from sarcoidosis peripheral blood mononuclear cells

Clinical and Experimental Immunology. 2007;150:460-68

Cross-reactive and species specific Mycobacterium tuberculosis antigens in the immunoprofile of Schaumann bodies: a major clue to the etiology of sarcoidosis

- Ang Sc and Moscovic EA. Histolo Histopathol. 1996;11:125-34
- Immunohistochemistry was performed on formalin-fixed tissue from 8 patients with sarcoidosis using panels of antibodies against mycobacterial antigens
- Schaumann bodies stained intensely for both mycobacterial antigens and host cytoplasmic proteins

Moller DR. Proc Am Thorac Soc. 2007; 4:465-68

Case 1  Summer 2002

- 36 y/o white male presented with non-productive cough, chest pain, dyspnea

- Mediastinoscopic lymph node biopsy showed non-caseating granulomatosus inflammation, no AFB or fungus

- Anaerobic culture <1+ Propionibacterium species

Malignant Granulomas
Propionibacterium acnes

- Investigated bacterial growth (aerobic & anaerobic) from lymph nodes removed under aseptic conditions
- No aerobic bacteria were isolated
- Propionibacterium acnes isolated from 75% of nodes from sarcoid patients
- No other bacteria or fungi grew in culture

- P. acnes cultured from 31/40 (78%) of sarcoidosis lymph nodes, vs. 38/180 (21%) of lymph nodes from control lymph nodes, p<0.001.
- % positivity correlated strongly with the extent of granulomas within the node
  - >75% of node granulomas: 100% cx +
  - 50-75% of node granulomas: 78% cx +
  - <50% of node granulomas: 67% cx +

Quantitative Analysis of Mycobacterial and Propionibacterial DNA in Lymph Nodes of Japanese and European Patients with Sarcoidosis

Yoshinobu Eishi,1,2 Moritaka Sugi,1 Hiroshi Ishige,1 Daivuke Kobayashi,1 Tetsuo Yamada,1 Takeshi Takeda,1 Francesco-Takahashi,2 Masaaki Kodai,1 Shigeho Kuchibe,1 Uichiro Guima,1 Josse Guima,1 Giuseppe Rizzatti,2 Marcello Cambrecia,3 Ronald J. Iosso,5 Andrew G. V. Holmes,1 Ono F. Ishimura,5 and Masayuki Ito1

Department of Human Pathology, School of Medicine, Tokai Medical and Dental University,1 Department of Pathology, Graduate School of Medicine, Kyoto University,2 Department of Internal Medicine, Nagasaki Medical School,3 Tokyo, and First Department of Internal Medicine, School of Medicine, Kumamoto University,4 Psychiatry and Allergology, Bioreduction: Biotechnological Research,5 Guy’s, St Thomas’, and St Bartholomew’s Hospitals, London, United Kingdom, and Department of Pathology, School of Medicine, University of Southern California, Los Angeles, California 900335

Received 22 March 2002; revised for re-publication 27 July 2003; accepted 9 September 2003

Molecular studies of Propionibacteria in sarcoidosis

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Positive samples/total samples</th>
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<tbody>
<tr>
<td>Eishi et al.</td>
<td>1999</td>
<td>15/15</td>
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<tr>
<td>Eishi et al.</td>
<td>2002</td>
<td>110/118</td>
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<td>Yamada et al.</td>
<td>2002</td>
<td>8/9</td>
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<tr>
<td>Gazouli et al.</td>
<td>2002</td>
<td>20/46</td>
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In situ hybridization using *P. acnes* specific probe

Animal Studies

Mouse *P. acnes*  
Human sarcoid

-lymphocytic alveolitis

-CD4+ T-cells

-polarized Th1 cytokine profile

Figures 1. CARDP with HRP-conjugated antibody for *P. acnes* in a formalin-fixed and paraffin-embedded tissue section of the lung from a rat given an intranasal infection of *P. acnes* (ATCC 49919). Dark brown dots and large brown aggregates are seen in the cytoplasm of medium to large size, b) neighbouring hepatocytes but not signals

-lymphocytic alveolitis

-CD4+ T-cells

-polarized Th1 cytokine profile
Table 2. Clinical Features in 12 Cases at the Onset of the Study and Course During Minocycline Therapy

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Race</th>
<th>Presenting Symptoms</th>
<th>Course and Current Status</th>
<th>Duration of Treatment (wk)</th>
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<tbody>
<tr>
<td>1</td>
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<td>White</td>
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<td>DM &amp; chest pain; lymphadenopathy</td>
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<td>DM &amp; chest pain; lymphadenopathy</td>
<td>72</td>
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<tr>
<td>3</td>
<td>M</td>
<td>13</td>
<td>White</td>
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<td>DM &amp; chest pain; lymphadenopathy</td>
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<td>DM &amp; chest pain; lymphadenopathy</td>
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<td>M</td>
<td>15</td>
<td>White</td>
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<td>DM &amp; chest pain; lymphadenopathy</td>
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<td>DM &amp; chest pain; lymphadenopathy</td>
<td>72</td>
</tr>
</tbody>
</table>

*DM indicates disease activity.*

*Pleural fluid analysis was normal.*

*Case 2 February 2003*

- 26 year-old AA female presented with pleuritic chest pain
- Chest CT: mediastinal and hilar adenopathy, multiple parenchymal masses
- Transthoracic needle biopsy
- Pathology: non-caseating granulomas, special stains showed no organisms
Case 2 August 2004
- Cutaneous lesions
- Skin Biopsy:
  - Epithelioid granulomas, little necrosis. No foreign material upon examination with polarized light. PAS stain negative

Case 2 January 2005
- Symptoms: wheezing and dyspnea
- Spirometry:
  - FVC: 2.72 (down from 3.36L)
  - FEV1: 2.07 (down from 2.28L)
  - DLCO: 19.0 (down from 22.8 ml/mmHg/min)
- Exam:
  - Papules and plaques on neck, face, trunk, arms, legs
- Lab:
  - Elevated LFTs, elevated ACE

Case 2 January 2005
- PA AND LATERAL CHEST DICTATED: 2/15/05
  REASON FOR EXAM: Sarcoid. No prior chest exams are available for comparison. The heart is not enlarged. There is evidence for some mediastinal adenopathy and considerable bilateral interstitial and alveolar infiltrates are present with some coalescence. The findings are consistent with the diagnosis of sarcoid with a moderately advanced parenchymal involvement. Without old films for comparison, it is impossible to exclude acute infiltrates or superimposed infection.
  IMPRESSION: Findings are most consistent with sarcoid with mediastinal and hilar adenopathy together with diffuse parenchymal disease.

Case 2 January 2005
- Minocycline and topical steroids

Case 2 January 2005
- Minocycline and topical steroids

Case 2 January 2006
- Minocycline and topical steroids

Case 2 January 2006
- Minocycline and topical steroids
Case 2 Pulmonary Function

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<tr>
<th>Date</th>
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<th>FEV1</th>
<th>DLCO</th>
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<td>2.28</td>
<td>19.0</td>
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<td>3.77</td>
<td>2.68</td>
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<td>8/15/06</td>
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Case 2

Labs pre/post minocycline

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<tr>
<th>Date</th>
<th>ACE</th>
<th>TP</th>
<th>Alk Phos</th>
<th>GGT</th>
<th>ALC</th>
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<td>557</td>
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<td>8.4</td>
<td>286</td>
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<tr>
<td>8/15/06</td>
<td>50</td>
<td>4</td>
<td>114</td>
<td>196</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Toxicity of minocycline

Infectious causes or contributors to “non-infectious” human diseases

- Tropheryma whipplei > Whipple's Dz
- Helicobacter pylori > Gastric ulcers
- Bacteria > Sarcoidosis