Objectives

1) Review epidemiologic and clinical data that support that RA may be initiated in the lung

2) Review lung anatomy and physiology to understand how the lung may be a site of generation of autoimmunity

3) Discuss necessary steps to prove that the lung is a site of initiation of autoimmunity in RA, with presentation of data from our own studies in this area.

Epidemiologic and clinical evidence that RA is initiated in the lung

Inhaled risk factors for RA

Tobacco smoke  Klareskog et al 2008

Dust  Oliver et al Scan J Rheumatol 2006

Pollution  Hart et al Environ Health Perspect 2009

It is possible that these factors lead to initial injury/inflammation and autoimmunity in the lungs
Lung disease and RA-related autoimmunity are present soon after and preceding joint disease


4 RF/CCP+ subjects with ILD and no IA at presentation
1 later developed IA classifiable as RA
Gizinski et al Clin Rheumatol 2009

74 CCP+ subjects with lung disease and no IA
3 later developed IA classifiable as RA
Fischer et al Abstract, ACR Annual Meeting 2010 (submitted)

Caveats

Do inhaled factors initiate autoimmunity in the lung, or do they travel thru the lung to cause organ injury elsewhere?
Example: smoking and bladder cancer – acrolein from tobacco toxic to the bladder

Are inhaled factors permissive for some other factor?
Example: smoking allows infection

Lung disease preceding joint disease may not mean that RA is initiated in the lungs - the lung may be an early target of circulating RA-related autoimmunity

Anatomy and physiology of the lung supports that the lung may be a site of generation of autoimmunity

Immunity in the lung

“BALT” Bronchial Associated Lymphatic Tissue
Localized response to infections
Generates IgA and other isotypes at mucosal surface
-these antibodies can become systemic

Reviewed in Randall TD. Adv Immunol 2010
BALT has been demonstrated in patients with RA and clinically-apparent lung disease

Rangel-Moreno et al J Clin Invest 2006

BALT in RA

Normal Lung

RA Lung

Hypothesis: The lung is a site of initiation of RA

Environmental factors

Inflammatory joint disease / RA

Initial generation of RA-related autoimmunity in the lung due to genetic + environmental interactions

Spread of autoimmunity from the lungs to the circulation ('Preclinical RA')

How to ‘prove’ that the lung is a site of initiation of RA-related autoimmunity?

1) Demonstrate the generation of RA-related autoimmunity in the lung in absence of and prior to joint disease

2) Identify factors that can drive the initiation of RA-related autoimmunity in the lung

3) Demonstrate mechanisms by-which RA-related autoimmunity may start in the lungs and then move to the joints

The lung may not be the site, or the only site, of initial generation of RA-related autoimmunity

Oral cavity, gut, GU, etc. -- each of these locations has its own mucosal-associated lymphatic tissue

But, proving that other sites are the origin of RA requires similar approaches.
A crucial part of demonstrating that RA starts in the lung (or any other site) is to identify and study individuals that are in the early phases of RA development.

How do we find individuals to study in real-time that are in the ‘preclinical’ period of RA development?

Phases of RA Development

- **Phase 1**: Genetic Risk
- **Phase 2**: Pre-Clinical Autoimmunity
- **Phase 3**: Clinical Disease

Studies of the Etiologies of Rheumatoid Arthritis (SERA)

Multi-site project based at the Univ of Colorado designed to understand the natural history of RA development through prospective evaluations of individuals at-risk for future RA.

PI’s: V. Michael Holers, Jill M. Norris

Subject enrollment sites:
- Denver, CO (University of Colorado)
- Los Angeles, CA (Cedars Sinai)
- Seattle, WA (Benaroya Institute)
- Omaha, NE (RAIN Network)
- Chicago, IL (University of Chicago)
- New York, NY (North Shore-Long Island)

Multiple collaborations
SERA: Lung Project

Central hypothesis: RA is initiated in the lung

Initial aims:
1) Demonstrate non-invasively that inflammatory lung abnormalities are present in preclinical RA
2) Demonstrate production of RA-related autoimmunity within the lung
   - Established RA
   - Preclinical RA

Airways abnormalities more common in Ab(+) subjects compared to Ab(-) controls
Demoruelle MK et al, Abstract 769

<table>
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<th>Autoantibody (-) Controls (N=15)</th>
<th>Autoantibody (+) Cases (N=42)</th>
<th>P-value</th>
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<tbody>
<tr>
<td>All Subjects</td>
<td>33% (5)</td>
<td>76% (32)</td>
<td>0.005</td>
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<tr>
<td>Never smokers</td>
<td>33% (4/12)</td>
<td>73% (19/26)</td>
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</table>

Airways abnormalities = bronchial wall thickening, bronchiectasis, centrilobular opacities (bronchiolitis) and air trapping

3/42 of Ab(+) cases have developed classifiable RA ~12 months after their participation in the lung study

Bronchial wall thickening

Autoantibody (+) Case

Autoantibody (-) Control

Airways abnormalities are similar in Ab(+) subjects without IA, and patients with early RA (<8 months joint symptoms)
Demoruelle MK et al, Abstract 769

<table>
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<tr>
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<th>Autoantibody (+) Cases (N=42)</th>
<th>Early RA (N=12)</th>
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<tr>
<td>All Subjects</td>
<td>76% (32)</td>
<td>92%</td>
<td>0.421</td>
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<tr>
<td>Never smokers</td>
<td>73% (19/26)</td>
<td>86% (6/7)</td>
<td>0.652</td>
</tr>
</tbody>
</table>
Lung biopsy studies of patients with established RA show that similar HRCT findings of airway thickening correlate with inflammatory changes and even BALT. Brown KK, Proc Am Thorac Soc 2007; Rangel-Moreno, J Clin Invest 2006

Could HRCT-airways disease indicate BALT in these Ab(+) subjects?

Aim 2:
Evaluate proteins and autoantibodies in lung samples from patients with established RA and controls

Total and citrullinated protein levels are higher in bronchoalveolar lavage (BAL) samples of patients with established RA and ILD

- 5 patients with established RA and ILD
- 5 patients with scleroderma and ILD
- 32 healthy controls (12 non-smokers, 20 smokers)

RA-related autoantibodies are elevated in BAL from patients with RA and lung disease compared to controls

How RA-related autoantibodies are getting into BAL and sputa?
Translocation from blood through inflamed lung tissue?
Generation within the lung?

Findings from Rangel-Moreno suggest that generation within BALT possible

Needs evaluation with comparative studies of BAL/sputa and blood, and tissue studies which are underway

Anti-CCP2 levels are elevated in serum and induced sputa in patients with early RA (<8 months of symptoms) compared to controls

Translocation from blood through inflamed lung tissue?
Generation within the lung?
Findings from Rangel-Moreno suggest that generation within BALT possible

Needs evaluation with comparative studies of BAL/sputa and blood, and tissue studies which are underway
In summary, these SERA lung studies show:

1) Lungs findings consistent with inflammation in Ab(+) individuals with RA, in most cases in absence of smoking. Supporting that the lungs are at least inflamed in preclinical RA and may be a site of initiation of RA.

2) RA-related autoantibodies are detectable in lung biospecimens from patients with established RA. Going forward, these methods will be used in preclinical studies to study generation of RA-related autoimmunity in the lung.

How to ‘prove’ that the lung is a site of initiation of RA-related autoimmunity?

1a) Demonstrate that the lungs are abnormal in preclinical RA
1b) Demonstrate that RA-related autoimmunity detectable in lung biospecimens in established RA
2) Demonstrate the generation of RA-related autoimmunity in the lung in absence of and prior to joint disease
3) Identify factors that can drive the initiation of RA-related autoimmunity in the lung
4) Demonstrate mechanisms by which RA-related autoimmunity may start in the lungs and then move to the joints: immune complexes? cells? antigen targeting of joint tissue?

What specific factors lead to RA-related autoimmunity in the lung?

Does process start with the generation of autoantigens?
If yes, what generates these autoantigens?

Smoking leads to increased PAD and cit-proteins in the lung
Makrygiannakis et al, Ann Rheum Disease 2008

Additional work from this group, including identification of similar antigens in the lungs and in the joints in established RA
ACR Abstracts 2176, 2179

What specific factors lead to RA-related autoimmunity in the lung?

Certain infections may lead to cit-proteins, such as P ging Venables, Lundberg and colleagues

Could infection generate autoantigens in the lung?

What is the infection?
Where does the infection come from?
Possible connection between oral and lung inflammation?

How long does an infection have to be present to initiate RA?
Are there other factors that generate inflammation and autoimmunity in the lung?

Summary

1) Environmental and clinical factors, and lung biology suggest the lung may be a site of initial generation of RA-related autoimmunity

2) Multiple steps are needed to prove that RA-related autoimmunity is generated in the lung (or any site)

3) To take these steps, crucial to identify and study in real-time subjects that are in the preclinical phases of RA

Studies in the SERA preclinical RA cohort have already yielded important data regarding the lung in preclinical RA

1) Identify the mechanisms of disease development
2) Prevention

Environmental factors

Initial generation of RA-related autoimmunity in the lung due to genetic + environmental interactions

Inflammatory joint disease/symptomatic RA

Spread of autoimmunity from the lungs to the circulation ('Preclinical RA')
Studies of the Etiologies of Rheumatoid Arthritis (SERA)

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