Human microbiome
and Systemic Lupus Erythematosus

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Disclosures

Research funding from

NIH
Lupus Research Institute
The Judith and Stuart Colton Foundation
Learning Objectives

1. Review basic principles regarding the role of intestinal commensals in immune maturation.

2. Discuss how commensal bacteria with pathologic properties can trigger inflammatory and autoimmune disease in susceptible individuals.

3. Understand emerging evidence of hyperactive IgA responses and microbial dysbioses in clinical SLE.
We all contain a Zoo of commensal bacteria

- Our intestines are home to a total of \(10^{10}-10^{14}\) microbial cells, 1000 or more different types of bacteria in our intestines.

  ~ 10 times more than the number of host cells.

  100 times more microbial genes than human genes.

- More than 30% cannot even be cultured in the lab.

- Is the microbiome the strongest environmental influence?
Major functions of intestinal microbiome

- **Nutrition**
  - synthesize vitamins

- **Metabolism**
  - process complex carbohydrates and make substances like butyrate
  - digest and release single chain Fatty Acids
  - catabolize of Tryptophan, substrate for 2,3 IDO for tolerance

- **Shape the immune system**
  - induction of TGFb/IL-10 for active tolerance
  - Induction of Th17 cells, IL-22 and TNFa-secreting cells

Are there symbionts/commensals with pathogenic properties?

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Pathogenic bacteria- inherently cause pathogenic tissue injury
Other commensals that are symbiotic with the host, and can initiate protective immune responses, but in predisposed hosts or compromised/injured tissue can contribute to inflammatory disease or autoimmune disease

Hypothesis: SLE have sets of pathobiont bacteria that contribute at different phases of the SLE disease process (and/or a decrease in protective (anti-inflammatory) bacterial strains
• SLE is a chronic, multisystem autoimmune disease\textsuperscript{1-3}
  – Hallmark of B cell hyperactivity and autoantibodies to DNA/RNA related structures and/or phospholipids due specific breaches in immune tolerance
  – Diverse clinical manifestations are the result of inflammation in affected organ systems\textsuperscript{1}
  – Potentially life threatening when major organs are affected\textsuperscript{2,3}
  – Waxing and waning disease activity\textsuperscript{4}

• SLE demographics
  - Estimated 1.5 million cases of lupus in US
  - Prevalence of 17 to 48 per 100,000 population

• SLE patient profile:
  – Nine out of 10 cases in women\textsuperscript{2}; often more severe in men\textsuperscript{5}
  – Most prevalent in women 14 to 50 years of age\textsuperscript{6}
  – More common and severe among nonwhite populations\textsuperscript{5}

SLE is a chronic systemic autoimmune disease with heterogeneous clinical features and organ system involvement.

### Table 1. Revised criteria of the American College of Rheumatology (formerly American Rheumatism Association) for the classification of systemic lupus erythematosus.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Malar rash</td>
<td>Fixed erythema, flat or raised, over the malar eminences tending to spare the nasolabial folds</td>
</tr>
<tr>
<td>Discoid rash</td>
<td>Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring can occur in older lesions</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation</td>
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<tr>
<td>Oral ulcers</td>
<td>Oral or nasopharyngeal ulceration, usually painless, observed by a physician or evidence of pleural effusion or Pericarditis: documented by ECG or rub or evidence of pericardial effusion</td>
</tr>
<tr>
<td>Arthritis</td>
<td>Non-erosive arthritis involving two or more peripheral joints, characterised by tenderness, swelling, or effusion</td>
</tr>
<tr>
<td>Serositis</td>
<td>Pleuritis: convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion or Pericarditis: documented by ECG or rub or evidence of pericardial effusion</td>
</tr>
<tr>
<td>Renal disorder</td>
<td>Persistent proteinuria greater than 0.5 g/day or greater than 3+ if quantification not performed or cellular casts: can be red cell, haemoglobin, granular, tubular or mixed</td>
</tr>
<tr>
<td>Neurological disorder</td>
<td>Seizures: in the absence of offending drugs or known metabolic derangements, e.g., uraemia, ketoacidosis or electrolyte imbalance or Psychosis: in the absence of offending drugs or known metabolic derangements, e.g., uraemia, ketoacidosis or electrolyte imbalance</td>
</tr>
<tr>
<td>Haematological disorder</td>
<td>Haemolytic anaemia: with reticulocytosis or Leucopenia: less than 4000/mm³ or Lymphopenia: less than 1500/mm³ or Thrombocytopenia: less than 1500/mm³</td>
</tr>
<tr>
<td>Immunological disorder</td>
<td>Anti-DNA: antibody to native DNA in abnormal titre or Anti-Sm: presence of antibody to Sm nuclear antigen or (c) Positive finding of antiphospholipid antibodies based on: (i) an abnormal serum level of IgG or IgM anticardiolipin antibodies; (ii) a positive test for lupus anticoagulant using a standard method; or (iii) a false-positive test for at least 6 months and confirmed by Treponema pallidum immobilisation or fluorescent treponemal antibody absorption test</td>
</tr>
<tr>
<td>Positive antinuclear antibody</td>
<td>An abnormal titre of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time in the absence of drugs</td>
</tr>
</tbody>
</table>

**4/11 criteria at some time**

Multi-organ involvement in SLE.

Challenges for microbiome studies in SLE

SLE is uncommon – about 1:1000-2000

Greater than 30 different genetic susceptibility factors
  diverse autoantibody/immune abnormalities
  diverse clinical features in different patients
Great heterogeneity of SLE patients

Unlike IBD- in SLE colitis/enteritis is very rare – problem is not in bowel (or is it?)

SLE diagnosis is based on 11 criteria documented in the past or present

DISEASE MAY BE INACTIVE WHEN A PATIENT IS ENROLLED
  Are inactive patients different than healthy individuals
BUT IF DISEASE IS ACTIVE IT REQUIRES MEDICATION (often by mouth)

Logistics of stool collection may disallow collection during disease flare
Anti-nuclear antibodies: Hallmark but not a single type of antibody
Hypothesis: Disease triggers and flares involve microbiome dysbiosis
Initial focus: Adult Female SLE patient in a cross-sectional survey

To compare groups of SLE patients-
SLE Disease Activity Index (SLEDAI)

-a composite index that factors all major SLE disease features in a continuous scale
SLE and the Microbiome

• Protocol: Plasma/sera, PBMC, Urine, DNA, RNA, Stool
• Enroll at three NYU sites: Bellevue, CMC, HJD SLE patients and controls
  - gender, age, ethnicity, co-morbidities, medications, diet?
• Clinical features (e.g., renal vs non-renal vs thromboembolic)
• By immune phenotype (serum isotype levels and autoAbs, IFN signature and others)
• Fecal IgA surveys (levels and specificity)
• Intestinal Microbiome
• In vivo immune recognition of some microbiota – IgA coated
• From cross-sectional studies to Next steps
  - New onset
  - Longitudinal
  - Intervention
Bacterial 16s rRNA PCR amplification and sequencing

Fecal sample

Modified from Morgan X C and Huttenhower. C PLOS Comput Biology 2012
Chao1 alpha diversity of Operational Taxonic Units (OTU)

• OTU are assigned based on 16S ribosomal gene sequences from V3-V4 amplimers, and NGS on the MiSeq instrument.

• alpha diversity measures how evenly the abundance of community members is distributed across OTUs.

• Higher alpha diversity indicates more evenly distributed communities, with maximum attained when all OTUs have the same number of representatives.

• Compared to healthy females, SLE subjects are less diverse by Chao1 (P<0.04).
Beta Diversity of OTU

• Beta diversity estimates the difference between communities of particular OTU (quasi-species)

Operational Taxonomic Unit (OTU)
quasi-species determination based on 16S genomic sequence
Principal Component Analysis of Jensen-Shannon Divergence distances in intestinal bacterial communities

ADONIS estimates for Jensen-Shannon Divergence

$P < 0.019$ *
Different sets of SLE patients have distinct patterns of inflammatory cytokines and chemokines

Can cytokine/chemokine patterns define distinct SLE subsets?

Could these patient subsets benefit from therapies that target their cytokine/chemokine disease drivers?

Could SLE patient subsets/defined by cytokine /chemokines be linked to distinct microbiomes?

Preliminary results with an enriched microbiome in SLE IFN type I compared to SLE IFN low
Could intestinal pathobionts contribute to B cell abnormalities in SLE pathogenesis?

• SLE is associated with B cell abnormalities, hypergammaglobulinemia, immune complexes and IgG autoantibodies?

• Have abnormalities in IgA regulation in SLE been overlooked?
Most of the antibodies in our bodies are IgA. More than all other isotypes combined!
3-5 grams secreted into our gut each day.

In germ-free mice, little or no intestinal IgA (or serum IgA)

Could distinct intestinal bacterial taxa be inducing IgA responses?

Flavell and coworkers argue that most bacteria-specific IgA in the gut-associated lymphoid tissue (GALT) arise from T-cell dependent germinal center reactions (1).

Can we identify the bacterial taxa that are targets of immune responses in intestines of SLE patients?
How to identify microbes that directly simulate gut-associated lymphoid tissue to induce autoimmunity?

Pathobiont
Targeted Plasma cells

Secretory dimeric IgA

GALT
Recovery of *in vivo* IgA coated bacteria

1. **Fecal sample**
2. **Supernatant bacteria**
   - Anti IgA-PE staining and binding with Anti PE beads
   - MACS column separation
   - MACS +
   - MACS -
   - 16s gene sequencing
3. **DNA 16s**
4. **MACS FACS +**
5. **FACS purification**
6. **MACS FACS +**
7. **16s gene sequencing**
MACS/FACS strategy enables separation into IgA coated and IgA-not coated bacteria

Only a proportion of intestinal bacteria are recognized by our B cells and their antibodies

Doua Azzouz
Principal Component Analysis of IgA coated Taxa
by Jensen-Shannon Divergence distances

PCo1 vs PCo 2

ADONIS p-value: 0.01
Is the dysbiosis associated with immune hyperactivity based on serum Ig levels?

P=0.0017  P= 0.0016  P=0.1140

Healthy N=20, SLE N=132

Serum IgM has a trend to lower in SLE

All IgG values above 5 mg/mL = 500 mg/dL

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SLE patients commonly display dysbiosis in intestinal microbiome.

Microbial diversity is reduced compared to healthy adults, and bacterial taxa (and phylogenetic assignment) diverge significantly from healthy controls.

Dysbiosis is more severe in SLE patients with more severe disease.

In vivo IgA coating in vivo suggests commensal bacteria stimulate the adaptive immune systems of SLE patients.
Acknowledgements

NYU SOM

Laboratory of B cell Immunobiology
Doua Azzouz, PhD
Hanane El Bannoudi, PhD
Lelise Getsu

NYU
Pam Rosenthal MD

Dan Littman, MD PhD

Bioinformatics
Alex Alekseyenko. PhD
(now at MUSC)

Next Generation DNA Sequence Analysis
Adriana Heguy,PhD (NYU GTC)

Clinical Director
Jill Buyon, MD

Visit our website
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