Epigenetics and Autoimmune Diseases: Potential Targets for Therapeutic Intervention

Beverly D. Lyn-Cook, Ph.D.
Division of Biochemical Toxicology

Epigenetics and Selected Biomarkers of Organ Toxicity

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Innovative Science To Improve Public Health
Human Autoimmune Diseases (AD)

- These diseases are defined as diseases in which immune responses to specific self-antigens contribute to ongoing tissue damage. The damage is mediated by autoantibodies and autoreactive T cells.

- Tissue – specific
- Systemic – involving multiple tissues

Four Phases of the Development of Autoimmune Diseases

<table>
<thead>
<tr>
<th>Susceptibility</th>
<th>Initiation</th>
<th>Propagation</th>
<th>Regulation</th>
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Factors Contributing to Autoimmune Diseases

Environmental and genetic factors contribute to an individual susceptibility to develop autoimmune diseases. Different immune cells response to pathogens.
Autoimmune Diseases and Target Organs or Components

<table>
<thead>
<tr>
<th>Disease</th>
<th>Target</th>
</tr>
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<tbody>
<tr>
<td>Multiple sclerosis</td>
<td>Central nervous system myelin</td>
</tr>
<tr>
<td>Type I diabetes</td>
<td>Beta-islet cells of the pancreas</td>
</tr>
<tr>
<td>SLE</td>
<td>Cell Nucleus</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Joints</td>
</tr>
<tr>
<td>Graves’ disease</td>
<td>Thyroid</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>Liver</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>Skin</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>Acetylcholine receptors</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>Joints</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>Nuclei, heart, lungs, kidney, gastrointestinal tract</td>
</tr>
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</table>
What is the evidence to support a role for epigenetics in the development of Autoimmunity?

Genetics cannot fully explain the hereditary patterns of autoimmune diseases, although genome-wide association studies have shown genetic polymorphisms can account for about 20%.

- Studies from Monozygotic Twins
- Animals Models
- Geographic and occupational clustering, suggesting environmental factors
Incidence of Autoimmune Diseases in Male and Female
Systemic lupus erythematosus

An autoimmune disease that affects many parts of the body. The immune system attacks the body’s cell and tissue resulting in inflammation and tissue damage. The immune system produces antibodies against itself.
Ethnicity Differences in SLE

- African American women are 4X more likely than European American women to develop lupus.

- Latino and Asian-American women are twice as likely as European American women to develop lupus.
Sex and Ethnic Disparities

Evidence for epigenetic mechanisms in systemic lupus erythematosus

- DNA Methylation
  - Global hypomethylation of B and T lymphocytes
  - Altered DNMT expression in SLE CD4+ T cells
  - MAPK/ERK overexpression in SLE results in impaired DNMT1 expression
  - Cytokine genes: demetylation (IL-4,6,10,13)
  - Demethylation of HERV element LINE-1 in CD4+, CD8+ T cells and B lymphocytes
  - Increased methylation of the death receptor 3 gene
  - Demethylation of co-stimulatory molecules (CD6,CD11A, CD40L, CD5)
Histone Modification

• Evidence in support of increased acetylation
• Histone modifications and autoantibody production
  • Acetylation motifs accelerate disease onset and severity
• Evidence in support of reduced acetylation
  • HDI downregulate IL-2,6 and 10, IFN-gamma in MRL/lpr mice
  • HDAC (Sirt 1) is overexpressed in MRL/pr CD4T lymphocytes
    – Suppression of Sirt 1 activity by anti-Sirt1 miRNA results in:
      – Elevation of H3 and H4 acetylation
      – Reduced anti-DNA titers, renal IgG deposition and renal pathology scores
miRNAs

• miRNAs are involved in DNA methylation
  • miR-126 regulates DNA methylation in SLE T cells
• Expression of miRNAs
  • miR-146a- abnormal activation of type I IFN/NFkB pathyways
  • miR-148a and miR-21- direct and indirect targeting of DNMT1
  • miR-203 is upregulated in RASF in a DNA methylation-dependent manner
  • miR-203 overexpression of IL-6 and matrix metalloprotease1
Objectives

• To determine the profile expression of specific innate and adaptive immune response genes using PCR array technology non-lupus and lupus PBMCs.

• To determine the promoter methylation profile of genes involved in cytokines biosynthesis in a select group of systemic lupus erythematosus patients compared to age-matched non-lupus patients.

• To determine the expression of miRNA 146a in lupus and non-lupus patients
Methods

• **Global DNA Methylation** Quantification Using a MethylFlash Methylated DNA Quantification kit (ELISA-based assay)

• **Promoter Methylation** Using the Human Cytokine Production EpiTect Methyl II Signature PCR Array

• **RT² Profiler PCR Array for** Human Common Cytokines and Innate & Adaptive Responses Genes

• **Real-time PCR**
RESULTS
Population

- Part of the LUPUS study at Brody School of Medicine at East Carolina University
- 160 Patients Diagnosed with SLE
  - 70 African American Women
  - 50 Caucasian American Women
  - 20 African American Men
  - 20 Caucasian American Men
- 160 aged and sex matched healthy volunteers
Whole Blood Collection

- Blood was collected by venipuncture of antecubital vein between 9:00 AM-12:00 PM

- Circadian Patterns
  - Collection
    - Same Time of Day
    - Same Day of week
  - PAXgene Blood RNA Tube
Previous studies

- Ethnic difference in DNMT3A expression, AA having higher levels
- No difference in DNMT1 expression
- Ethnic difference in DNMT3B, AA having higher levels

Ethnic Differences in DNA Methyltransferases Expression in Patients with Systemic Lupus Erythematosus.
Kenneth L Wiley, Edward Treadwell, Kayihura Manigaba, Beverly Word, Beverly D Lyn-Cook
# Cytokine Production DNA Methylation PCR Array

<table>
<thead>
<tr>
<th><strong>Cell Function Regulators:</strong></th>
<th>BCL10, BCL3, FOXP3, HMOX1, IL12A, MALT1, MAP3K7, SOD1, STAT5A, TRAF2, TRAF6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B Cell Function Regulators:</strong></td>
<td>BCL10, BCL3, INHA, INHBA, STAT5A</td>
</tr>
<tr>
<td><strong>Transcriptional Regulators:</strong></td>
<td>BCL10, BCL3, FOXP3, GATA3, IRF1, SMAD3, STAT5A;</td>
</tr>
<tr>
<td><strong>Translational Regulators:</strong></td>
<td>BCL3, IGF2BP2</td>
</tr>
<tr>
<td><strong>Environment &amp; Intracellular Stimuli Responses:</strong></td>
<td>BCL10, BCL3, ELA2, FOXP3, GATA3, HMOX1, IL12A, INHA, INHBA, LTB, MALT1, MYD88, NOD1, SMAD3, SOD1, STAT5A, TLR2</td>
</tr>
<tr>
<td><strong>Cytokine Production Signaling Molecules:</strong></td>
<td>BCL10, BCL3, FOXP3, HMOX1, INHA, INHBA, LTB, MALT1, MAP3K7, MYD88, NOD1, SMAD3, SOD1, STAT5A, TLR2, TRAF2, TRAF6</td>
</tr>
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</table>
Lupus Patients

Two genes, FoxP3 and Elane, showed methylation promoter changes in lupus patient with moderate disease activity scores.
FoxP3

- Member of the forkhead family of transcription factors
- Essential transcription factor for Treg cells
- FoxP3 is known to form complexes with histone acetyltransferases (Tip60 and p300) or HDACs (HDAC7)
- Complexes epigenetically modulate target gene expression via histone or FoxP3 acetylation or deacetylation.
Regulatory T-cells (Tregs)

- Guardians of peripheral tolerance
- SLE and RA arise due to a failure of immunological self-tolerance
- Foxp3 is a critical transcription factor for the development and function of Tregs
- Animals studies have shown that mutation and depletion of the Foxp3 gene results in fatal autoimmune lymphoproliferative disease
- In human, several mutations have been linked to immune dysregulation
Gene Expression Arrays
FoxP3 expression in lupus and non-lupus age-match PBMCs
miRNA 146a

• miRNA 146a is a negative regulator of the IFN pathway
• Inhibition of endogenous miR-146a increased induction of type 1 IFNs in PBMCs in lupus patients
• miRNA 146a has been identified as key player in innate immunity
Type I Interferons (IFNs)

- Major controllers of viral infections
- An “IFN signature” has recently been identified by GWAS studies in a majority of active lupus patients
- Induced type 1 IFN could promote expansion of autoreactive T cells
- SLE blood constitutes a DC-inducing environment (numerous agents activates DC, microbes, dying cells, cells of innate immune system, cells of the adaptive immune system)
- DC-inducing property of SLE sera is mediated through IFN-α
IFN-α

• IFN-α up-regulates CD38 and BAFF (B cell activating factor) on monocytes and mDCs.
• BAFF contributes to the survival of autoreactive B lymphocytes
• IFN-α can also be activated through TLR 9 and/or 7 dependent pathways
• INF-α induces the transcription of TLR-7 itself.
miRNA 146 expression in lupus and non-lupus age-matched controls.
IFN-\(\alpha/\)actin

\[ P=0.001 \]

Interferon-alpha expression in lupus and non-lupus PBMCs
miRNA 146a (low level of expression)

- Bioinformatics studies have identified a potential CpG island in the promoter of miRNA 146a.
- Previous studies have shown the epigenetic regulation of several miRNAs by IL-6 in malignant human cholangiocytes.
- Several studies, including our own, have shown significant increase in serum IL-6 in SLE patients.
- The relationship between IL-6 and miRNA 146 low levels is unknown.
Conclusion

• Foxp3 promoter showed an increase in methylation in lupus patients (hypermethylation), which correlated to decreased expression

• FoxP3 from GWAS studies has been recently indicated in autoimmune diseases

• miRNA 146a was significantly decreased in lupus patients
New Targets for Treatments?

- Antagonists for TLRs and IFNs (caution because of potential for life-threatening immune deficiencies)
- Manipulation of miRNA levels could lead to novel therapeutic strategies to combat SLE
- A study showed that when miRNA146a was introduced in PBMCs from SLE patients, the activation of the type I IFN pathway was notably reduced, by downregulation of several IFN-inducible genes.
Belimumumab (anti-BAFF)- Benlysta for Treatment of SLE

- Blocks a B cell survival factor, inducing B cell death
- Recently approved (3/9/2011) for the treatment of SLE

- 1ST DRUG APPROVED FOR LUPUS IN OVER 50 YRS
- 1ST BIOLOGIC APPROVED FOR LUPUS
SLE pathogenesis and treatment targets

- Lymphocyte signaling
  - small molecule inhibitors
  - TNF blockade
  - IL-6 blockade

- Autoantibodies
  - Immune complex
  - Proteasome inhibitors
  - Anti-B cell antibodies
  - TLR inhibitors
  - IFNα blockade

- BAFF inhibitors
  - mBAFF
  - sBAFF

- CTLA4-Ig
  - Abatacept

- mDC
  - BR3
  - TLR9

- pDC
  - IFNα

- PC
  - CD40
  - CD40L
  - B7.1/2

- mDC
  - B7.1/2
  - CD28

- TLR9
  - B7.1/2

- Proteasome inhibitors
  - Anti-B cell antibodies
  - TLR inhibitors
  - IFNα blockade

- Autoantibodies
  - Immune complex

- Lymphocyte signaling
  - small molecule inhibitors
  - TNF blockade
  - IL-6 blockade
### Agents Currently Undergoing Clinical Trails to Treat Lupus

<table>
<thead>
<tr>
<th>Drug</th>
<th>Developed by</th>
<th>Phase</th>
<th>Target</th>
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<tbody>
<tr>
<td>CellCept</td>
<td>Roche</td>
<td>3</td>
<td>Inosine monophosphate dehydrogenase-1</td>
</tr>
<tr>
<td>Epratuzumab</td>
<td>UCB SA</td>
<td>3</td>
<td>CD20</td>
</tr>
<tr>
<td>LY2127399</td>
<td>Eli Lilly</td>
<td>3</td>
<td>BLyS</td>
</tr>
<tr>
<td>Atacicept</td>
<td>Merck KGaA</td>
<td>2/3</td>
<td>BLyS, APRIL</td>
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<tr>
<td>Ocrenica</td>
<td>Bristol-Myers Squibb</td>
<td>2/3</td>
<td>CD80, CD86</td>
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<tr>
<td>A-623</td>
<td>Anthera Pharmaceuticals</td>
<td>2b</td>
<td>BLyS</td>
</tr>
<tr>
<td>Lupuzor</td>
<td>Cephalon</td>
<td>2</td>
<td>T lymphocytes</td>
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<tr>
<td>Laquinimod</td>
<td>Teva</td>
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<td>T lymphocytes</td>
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<tr>
<td>MEDI-545</td>
<td>AstraZeneca</td>
<td>2</td>
<td>Interferon-alpha</td>
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<tr>
<td>Rontalizumab</td>
<td>Roche</td>
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<td>Interferon-alpha</td>
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<td>IFNalpha-Kinoid</td>
<td>Neovacs</td>
<td>1/2</td>
<td>Interferon-alpha</td>
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Chenghui Xie, PhD
Jarren Oates
Edward Treadwell, MD
Beverly Word
George Hammons, PhD
Kenneth Wiley, PhD
Pathology
FDA Office of Women’s Health