Presentation overview

• Autoimmune disease
• Role of epigenetics
• Example: Toxicant-induced epigenetic changes and autoimmune disease
Overview of autoimmune disease

- Immune attack against self antigens
- Impact ~5-9% of the US population
- Disproportionally affect females
- ~80-100 diseases
  - Lupus, Type I diabetes, inflammatory bowel diseases, Rheumatoid arthritis, Multiple sclerosis
- Classified based on target organ pathology
- Share common underlying immunological mechanisms
  - CD4 T cell-driven diseases
- Causes are not known
  - Both genetic and environmental risk factors contribute to disease
Epigenetics

- Epigenetics literally means “above” genetics.
- It refers to external modifications to our DNA that turns genes on and off.
- These modifications do not change the DNA sequence, but affect how our cells “read” genes.

Image obtained from ibalifesciences.com
There are several ways DNA is modified.

One of the most commonly characterized is DNA methylation. **Methylation** or addition of a methyl group to cytosines in the DNA sequence; specifically the ones followed by a guanine or CpG prevents binding of transcription factors and certain genes from being expressed.

Some regions of DNA can be **unmethylated** which encourages transcription factor binding and expression of genes.

Regulated by our environment

Heritable and potentially reversible

Epigenetics and the environment

Your DNA contains the recipe for making proteins.

RNA reads the DNA like a chef reading a recipe and helps assemble proteins.

Environmental factors can modify cellular processes that dictate which proteins need to be suppressed or activated.

You interact with the environment.

<table>
<thead>
<tr>
<th>Environmental factors</th>
<th>DNA</th>
<th>RNA</th>
<th>Protein</th>
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</thead>
<tbody>
<tr>
<td>sunlight</td>
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<td>food</td>
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<td>drugs</td>
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## DNA methylation alterations in autoimmune disease

<table>
<thead>
<tr>
<th>Autoimmune disease</th>
<th>DNA methylation alteration</th>
<th>consequences</th>
<th>Examples of affected genes</th>
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<tbody>
<tr>
<td>Lupus</td>
<td>Hypomethylation</td>
<td>Gene activation</td>
<td>CD70, CD154, IL-4, IL-6, CD9, MMP9 <em>(cytokines and signaling molecules)</em></td>
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<tr>
<td>Lupus</td>
<td>hypermethylation</td>
<td>Gene silencing</td>
<td>RUNX3, folate biosynthesis genes, IL-2, foxp3 <em>(T reg generation)</em></td>
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<tr>
<td>Rheumatoid arthritis</td>
<td>hypomethylation</td>
<td>Gene activation</td>
<td>CD40L, IL-6, IL-1 <em>(cytokines and signaling molecules)</em></td>
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<tr>
<td>Type I diabetes</td>
<td>hypermethylation</td>
<td>Gene silencing</td>
<td>Insulin, foxp3 <em>(T reg generation, insulin production in pancreatic cells)</em></td>
</tr>
</tbody>
</table>
DNA “hypomethylation” and autoimmune disease
DNA “hypomethylation” and autoimmune disease

Environmental factor

Promoter region

CD4 T cell gene

Methylation

Transcriptional repression

CH$_3$ CH$_3$

CGTAT CGTAA

Environmental factor
DNA “hypomethylation” and autoimmune disease

- Demethylation
- Transcription
- Translation

Environmental factor

Promoter region: CGTATCGTAA

Transcription factor

CD4 T cell gene

Autoimmune disease

- Cell cycle proteins (Cdkn1a)
- Adhesion molecules (CTLA-4, CD40L, CD70)
- Cytokines and Th differentiation (Th1, Th2, Th17, T_{REG})
Toxicants related to autoimmune disease and DNA methylation alterations

- Bisphenol A
- TCDD/AHR ligands
- Mercury
- Trichloroethylene
Toxicants related to autoimmune disease and DNA methylation alterations

- Bisphenol A
- TCDD/AHR ligands
- Mercury
- Trichloroethylene
  - CpG hypermethylation
Trichloroethylene (TCE) Exposure

- Factories and military sites
- Spills and surface water contamination
- Factory workers
- Spills seep into ground
- Well water contamination
- Vapor intrusion
- Superfund sites and hazardous waste sites
- Groundwater contamination
- TCE can be released into the air, water, and soil at places where it is produced or used.

What is TCE and how is it used?
- Industrial chemical solvent
- Used to make hydrofluorocarbon chemicals (e.g., refrigerants)
- Used as a solvent to remove grease from metal parts
- Used in some dry cleaning and consumer products (spot removers)

How are people exposed to TCE?
- Inhalation of indoor and outdoor air
- Drinking contaminated water
- Occupational exposure
- People living or working near waste sites experience higher levels of indoor TCE due to vapor intrusion

What are the health risks associated with TCE exposure in humans?
- TCE is a carcinogen (kidney and leukemia)
- Associated with congenital heart defects and low birth weight
- Immunotoxicity
- Altered numbers of peripheral blood CD4 T cells
- Associated with lupus, scleroderma, autoimmune hepatitis, Type I Diabetes
- Occupational TCE exposure associated with T cell-dependent hypersensitivity syndrome with exfoliative dermatitis, mucus membrane erosion, eosinophilia, and non-viral hepatitis

National Toxicology Program Summary of Trichloroethylene
Studying TCE-induced autoimmunity

**Susceptibility factors**
- female autoimmune-prone mice

**Dose**
- Occupationally relevant
- Environmentally relevant doses

**Route**
- Drinking water

**Timing**
- Developmental exposure vs. adult

**Co-exposures**
- High fat diet
- Other toxicants (mercury)

**Target organ toxicity**
- Liver, brain, gut, lung, etc.

**CD4⁺ T cells**

**Immune pathology**

**Autoimmunity**

**Mechanism?**
Mouse model of TCE-induced autoimmunity

- Adult female MRL mice exposed to TCE for 4-32 weeks in the drinking water at low-to-occupationally relevant doses
  - Expansion of effector memory CD4\(^+\) T cells (CD44\(^{hi}\)/CD62L\(^{lo}\))
  - Increased IFN-\(\gamma\) and IL-17
  - Autoimmune hepatitis

Trichloroethylene autoimmunity

- *Persistent and long-lasting effects suggest programming events: epigenetics*
  - Could TCE alter DNA methylation?
Genome-wide DNA methylation
Reduced Representation Bisulfite Sequencing (RRBS)

**40 weeks (adult exposure)**
**Gestation-early life** (37 weeks)

TCE (0 or 0.5 mg/ml)
DNA isolated from effector/memory CD4+ T cells activated ex vivo

RRBS is a method that selects for CpG rich Regions
CpG islands
Promoters
Regulatory elements

*Environ Epigenet. 2017 Jul;3(3)
**under review Frontiers in Immunology
Differentially methylated regions (DMRs) in regulatory elements: TCE vs. control

These proteins make up a transcription Factor complex called polycomb repressive Complex 2 (PRC2)

Under review: Frontiers in Immunology
Histones package DNA into nucleosomes

Polycomb Repressor Complex 2
- Acts as a transcriptional repressor
- Binds to H3K27
- EZH2 adds methyl groups to suppress gene expression
- Hypomethylated regions encourage PRC2 binding
Histones package DNA into nucleosomes.

Histone 3

DNA methyltransferase-1

<table>
<thead>
<tr>
<th>TCE (m g/ml)</th>
<th>fold change</th>
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<tbody>
<tr>
<td>resting</td>
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<tr>
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<tr>
<td>0.02</td>
<td>1.0</td>
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<tr>
<td>0.5</td>
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DNA methylation


EZH2 deficiency in CD4 cells
- Decreased regulatory T cells
- “excessive” IFN-γ,
- Proliferation/differentiation
- Enhanced cell survival

Autoimmune phenotype?
Conclusion

• Environmental toxicants may promote autoimmunity through alterations in DNA methylation in CD4 T cells.

• Epigenetic changes may represent an important pathway in autoimmune disease.
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