Role of Sex and Sex Hormones in Autoimmune Disease

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- No relevant financial with commercial interests with business lines related to this talk.

- Other non-relevant disclosures:
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Increase in autoimmune diseases over the past 5 decades

- NIH: 24 million Americans affected by autoimmune diseases
- 5th leading cause of morbidity in women

Potential reasons for increase in autoimmune diseases

- Decreased infectious diseases
- Increased industrialization – smoking/pollution
- Obesity
- Lowered sunlight exposure=lowered Vitamin D levels
- Changes in reproductive status/lowered number of pregnancies
Female prevalence in autoimmune diseases

- Hashimoto's disease
- Sjogren's syndrome
- Addison's disease
- Systemic sclerosis
- Systemic lupus erythematosus
- Primary biliary cirrhosis
- Autoimmune chronic hepatitis
- Graves' disease
- Antiphospholipid syndrome
- Idiopathic thrombocytopenic purpura
- Rheumatoid arthritis
- Myasthenia gravis
- Giant cell arteritis
- Pernicious anaemia
- Myositis
- Multiple sclerosis
- Vitiligo

As with many autoimmune diseases, there is a female predominance in MS of approximately 3:1

Evidence suggests that there is an increasing female prevalence of MS over the past 50 years, with a disproportionate increase in women

Orton, Lancet Neurology 2006
T cell subsets: Th1, Th2, Th17, regulatory T cells

The interplay of sex chromosomes, sex hormones, and immune responses influence sex differences in virologic control.

Genes on the X chromosome with the potential to influence immunocompetence

**Receptor & associated proteins**

- AR (Androgen receptor)
- A2AR (Angiotensin receptor)
- C5ORA (C5a receptors 1)
- CRH (Corticotropin-releasing factor receptor 1)
- CRHRR (CRH receptor 1)
- CYP1A1 (Cytochrome P450 family 1)
- ER (Estrogen receptor)
- ERK (ERK kinases)
- ERBB2 (ErbB2 receptor)
- ESR1 (Estradiol receptor alpha)
- ESR2 (Estradiol receptor beta)
- GPR57 (G protein-coupled receptor 57)
- GPRC2 (G protein-coupled receptor C2)
- HTR2A (Heterotrimeric G protein-coupled receptor 2a)
- MSHR (Mesenchymal-like hormone receptor)
- RASGRF1 (Ras guanyl-releasing factor 1)

**Transcriptional & translational control effectors**

- BACH1 (Basic helix-loop-helix protein)
- BACH2 (Basic helix-loop-helix protein 2)
- CREB (CAMP-responsive element binding protein)
- DBF4 (Double minute 4)
- DUSP6 (Dual specific protein kinase)
- EGR4 (EGR family member 4)
- ER (Estrogen receptor)
- ERK (ERK kinases)
- ESR1 (Estradiol receptor alpha)
- ESR2 (Estradiol receptor beta)
- FOS (Fos proto-oncogene)
- HIF1A (Hypoxia-inducible factor 1alpha)
- INSR (Insulin receptor)
- JUN (Jun proto-oncogene)
- KRAS (Kirsten rat sarcoma viral oncogene homolog)
- MAPK1 (Mitogen-activated protein kinase 1)
- MLL4 (Mitotic linker 4)
- NCOA3 (N-CoR family member 3)
- NR4A1 (Nuclear receptors 4A1)
- NOTCH1 (Notch homolog 1)
- PPARG (Peroxisome proliferator-activated receptor gamma)
- PRRA1 (PRR family member 1)
- PTEN (Phosphatase and tensin homolog)

**Sex Hormones: Overview**

**Male**

- Hypothalamus
  - Gnrh
  - Gnrh receptor
  - Pituatory
  - Anterior lobe (Leydig cells, Sertoli cells)
  - Testosterone, Inhibin

**Female**

- Hypothalamus
  - Gnrh
  - Gnrh receptor
  - Pituatory
  - Anterior lobe (Ovarian cells, Granulosa cells)
  - Estrogen, Inhibin

Fish, 2008
**Effects of 17-B estradiol on the immune system**

- CD4+ T cells
  - IL-4 and T2-cell responses (high oestrogen levels)
  - IFNγ and T1-cell responses (low oestrogen levels)
  - Tc [cell] numbers
  - CCR7 and CC19 expression
  - TNF production

- NK cells
  - Cytotoxic activity

- Monocytes and macrophages
  - IL-1, IL-6, and TNF production

- Dendritic cells
  - Differentiation of CD14+ DCs
  - IL-12 production
  - Expansion of IFN-γ-producing killer DCs from mature spleen DCs
  - Expression of IL-4, IL-8, and CCL2 by immature DCs

**Expression of estrogen receptors on immune cells**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Human</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESR1</td>
<td>ESR2</td>
</tr>
<tr>
<td>B cell</td>
<td>Yes (+)</td>
<td>Yes (+)</td>
</tr>
<tr>
<td>CD4+ T cell</td>
<td>Yes (+)</td>
<td>Yes (+)</td>
</tr>
<tr>
<td>CD8+ T cell</td>
<td>Yes (+)</td>
<td>Yes (+)</td>
</tr>
<tr>
<td>NK cell</td>
<td>Yes (+)</td>
<td>Yes (+)</td>
</tr>
<tr>
<td>Plasmacytoid DC</td>
<td>Yes (+)</td>
<td>Yes (+)</td>
</tr>
<tr>
<td>Monocyte</td>
<td>Yes (+)</td>
<td>Yes (+)</td>
</tr>
<tr>
<td>Monocyte-derived DC</td>
<td>Yes (+)</td>
<td>Yes (+)</td>
</tr>
<tr>
<td>Spleen DC</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Inflammatory DC (CNS)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Peritoneal macrophage</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>BM-derived macrophage</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hematopoietic stem cell</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* "Yes" indicates either RNA or protein expression, depending on the study. "No" indicates that the RNA or protein was queried but not found.

† Plus (+) marks indicate relative amounts of RNA determined using quantitative methods in one study (Ref. [13]).

يفّاقة، Cellular Immunology 2005
Effect of estrogens on immune cells

Pregnancy and autoimmune disease

Straub, Endocrine reviews, 2007
Why differential effects of estrogen levels (pregnancy vs. non-pregnancy) on the immune system?

- Evolution!
- Pregnancy shifts to Th2 (more tolerogenic immune response) to protect the fetus
- Non-pregnant shifts to Th1 to fight infections rapidly and preserve maternal/caregiver health
Sex hormone paradigm in MS

Bove and Chitnis, MSJ 2014

<table>
<thead>
<tr>
<th>Th1 cell mediated diseases</th>
<th>Th2/B cell mediated diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple sclerosis</td>
<td>Neuromyelitis Optica (NMO)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Systemic Lupus Erythematosus</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>Ulcerative colitis</td>
</tr>
<tr>
<td>Hashimoto’s thyroiditis</td>
<td>Grave’s disease</td>
</tr>
</tbody>
</table>
Hi/Lo Estrogen and the Th1-Th2 balance

Pregnancy: high E
Shift Th1 -> Th2

Flare:
- Graves'
- Myasthenia
- SLE

Improve:
- RA
- Hashimoto
- MS

Baseline, low levels E
- Favor Th1

MS: Influence of Pregnancy

MS Relapse Rates

Progesterone

Estrogens

0 12 24 38 Weeks

Delivery

Postpartum

Pregnancy
Estrogens increase BAFF levels and B cell maturation during pregnancy in NMO

Davoudi, Neurology, Neuroimmunology, Neuroinflammation, 2016
## Post-pubertal onset of most autoimmune diseases (childbearing years)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Typical age at onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>15-40 years</td>
</tr>
<tr>
<td>MS</td>
<td>20-35 years</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>20-30</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>20-40</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>15-40</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>20-40</td>
</tr>
</tbody>
</table>

Adapted from Beeson, *Am J. Medicine* 1983

## Puberty as a turning point for the initiation of MS

- Key risk exposures occur during adolescence
  - Obesity
  - Vitamin D
  - EBV
  - Migration studies

Chitnis, *Clinical Immunology* (review), 2011
Increased F:M ratio after age 11 in pediatric MS

Belman, Pediatrics 2016

80% of MS girls had onset post-menarche

80% of MS patients have pediatric-onset (<age 18 years) >80% are post-puberal

Chitnis, Annals Clinical Translational Neurology 2016
Early menarche heralds earlier onset of MS

- Early menarche (onset of menses) has been observed in women with adult-onset MS compared to healthy females.
- Relative risk reduction was 0.9 per each year of increased age at menarche (Ramagopalan, Eur J Neurol, 2009).
- With each one-year decrease in the age at menarche, the age at first symptoms was reduced by 1.16 years (D’Hooge, JN 2012).
- In our MS cohort, every year decrease in age at menarche was associated with a 0.65-year earlier MS onset (95% CI = [0.07-1.22], p = 0.027, N = 540) (Bove, Neurol. Genetics 2016).

Estrogen turns down “the AIRE”

Pearl Bakhru and Maureen A. Su

AIRE expression is necessary for thymic tolerance
Increasing evidence that obesity during adolescence is associated with a higher risk of MS in women (Munger, Neurology 2009; Munger MSJ 2013; Hedstrom, Neurology 2014)

- Weaker effects in men with MS (Munger MSJ 2013; Hedstrom, Neurology 2014)

- In children: one study found an effect of obesity in girls but not in boys; however, a smaller number of male cases may have limited the ability to detect an effect (Langer-Gould, Neurology 2013).  

- A higher BMI during adolescence may be associated with younger age at MS onset in women (Kavak, MSJ 2015)

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Obesity is characterized by a low-grade systemic inflammation due to the secretion of pro-inflammatory proteins in the blood (adipokines)

Adipose tissue is an active secretory organ modulating appetite, energy expenditure, insulin sensitivity, endocrine and reproductive system, inflammation and immunity.
Higher BMI in boys and girls: increases risk of MS

- MS boys overweight/obese:
  - 56% of pre-pubertal boys
  - 48% of pubertal/post-pubertal boys
- Odds-ratios for age-adjusted BMI on Risk of MS:
  - 2.28 (95% CI 0.95, 5.47) in pre-pubertal boys
  - 1.32 (95% CI 0.99, 1.77) in pubertal/post-pubertal boys

- MS girls overweight/obese
  - 26% of pre-menarcheal onset girls
  - 60% of post-menarcheal onset girls
- Odds-ratios for age-adjusted BMI on Risk of MS:
  - Pre-menarcheal onset girls- no effect of BMI on risk of MS.
  - In post-menarcheal girls, BMI z-score was associated with risk of MS with an unadjusted odds ratio of 1.68 (CI: 1.21, 2.34) and adjusted odds-ratio of 1.6 (CI: 1.12, 2.27).

Puberty and obesity affect age at onset of MS

- Age at onset of MS:
  - Girls: Younger age at menarche seems to mediate an effect of higher BMI.
  - Boys: Advanced sexual maturity (by Tanner staging) in boys was associated with younger onset of MS; these boys also had higher BMI than controls

Testosterone is converted to estradiol in the presence of Aromatases (increased in adipose tissue)

Increasing obesity and early menses worldwide
## Vitamin D and autoimmunity

### Risk factors for MS – also affect disease course

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Gender influence</th>
<th>Sex influence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D deficiency</td>
<td>★↑females</td>
<td><img src="image" alt="Interaction with estrogen receptor" /></td>
</tr>
<tr>
<td>Epstein-Barr virus exposure</td>
<td><img src="image" alt="?" /></td>
<td><img src="image" alt="?" /></td>
</tr>
<tr>
<td>Obesity</td>
<td>★↑males and females</td>
<td><img src="image" alt="?" /></td>
</tr>
<tr>
<td>Prenatal exposures?</td>
<td></td>
<td><img src="image" alt="?" /></td>
</tr>
</tbody>
</table>
Vitamin D
The “Sunshine Vitamin”

- 7-dehydrocholesterol is metabolized into Vitamin D3 by UVB skin exposure
- Vitamin D has anti-inflammatory effects and may reduce new MRI lesions in MS

- Genetic mutations which affect Vitamin D synthesis and function


Vitamin D effects on inhibition of CD4+ T cell proliferation and regulatory T cell formation is stronger in female mice

Correale, Journal of Immunology, 2010
Mechanisms of female immune “responsiveness” to Vitamin D

- Vitamin D degrading enzyme (CYP24A1) is lower in females than males
- Estradiol mediates down-regulation of CYP24A1, and increases expression of Vitamin D receptor
- Addition of 17-B estradiol reproduces Vitamin D “female” effects in male T cells
Males with autoimmune disease – Why?

Sex hormone paradigm in MS

<table>
<thead>
<tr>
<th>ESTROGEN</th>
<th>INFLAMMATION</th>
<th>NEURODEGENERATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL RANGES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(estradiol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PREGNANCY RANGES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(estriol)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TESTOSTERONE</th>
<th></th>
</tr>
</thead>
</table>

Bove and Chitnis, MSJ 2014
Androgens – immune regulation

Lowered immune responses to influenza virus in males vs. females
Association with levels of testosterone

Furman, PNAS 2014
Testosterone levels are low in half of MS men

40% of men have hypogonadal levels of testosterone (<300ng/ml)

No compensatory rise in LH implies impaired hypothalamic regulation

<table>
<thead>
<tr>
<th>AGE GROUP (Years)</th>
<th>N</th>
<th>T (ng/dl) Mean (SD)</th>
<th>MIN</th>
<th>MAX</th>
<th>% with T &lt; 300</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-29</td>
<td>13</td>
<td>374 (168)</td>
<td>167</td>
<td>651</td>
<td>46</td>
</tr>
<tr>
<td>30-34</td>
<td>20</td>
<td>328 (99)</td>
<td>133</td>
<td>513</td>
<td>40</td>
</tr>
<tr>
<td>35-39</td>
<td>24</td>
<td>352 (130)</td>
<td>142</td>
<td>646</td>
<td>37</td>
</tr>
<tr>
<td>40-44</td>
<td>12</td>
<td>345 (108)</td>
<td>166</td>
<td>517</td>
<td>33</td>
</tr>
<tr>
<td>45-49</td>
<td>12</td>
<td>375 (125)</td>
<td>237</td>
<td>671</td>
<td>42</td>
</tr>
<tr>
<td>50-54</td>
<td>6</td>
<td>254 (159)</td>
<td>49</td>
<td>472</td>
<td>67</td>
</tr>
<tr>
<td>55-59</td>
<td>8</td>
<td>297 (94)</td>
<td>126</td>
<td>450</td>
<td>50</td>
</tr>
<tr>
<td>60+</td>
<td>1</td>
<td>395</td>
<td>395</td>
<td>395</td>
<td>0</td>
</tr>
<tr>
<td>ALL</td>
<td>96</td>
<td>342 (126)</td>
<td>49</td>
<td>671</td>
<td>41%</td>
</tr>
</tbody>
</table>

Bove, MSJ 2014

Sex hormone paradigm in MS

Gillies and McArthur, Pharm. Reviews 2010
Ratio of 2nd:4th digit linked to in utero testosterone levels and chondrocyte gene expression

Zheng and Cohn, PNAS 2011

2D:4D a proxy measure for prenatal androgen exposure

Reflects low prenatal androgen levels in males with MS vs. non-MS

Mean (SD) 2D:4D ratio was higher in MS (0.9546 (0.04)) than in non-MS subjects (0.9456 (0.03)) (p=0.038)

Bove et al, Neurology 2015
Summary

- Autoimmune diseases are female prevalent
- X-chromosome carries immune-related genes
- Estrogens proinflammatory; Testosterone anti-inflammatory
- Estrogen at hi/lo doses has differential effects on immunity
- Pregnancy levels (hi) estrogen is protective in Th1 but not Th2/B cell mediated diseases
- Sexually dimorphic effects of Vitamin D and obesity
- Puberty is a key initiation point for T cell mediated autoimmune diseases
- Low testosterone levels associated with MS in males

Thank you!

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