Impact of Environmental Factors on MS Phenotype

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Plan of Presentation

- The hygiene hypothesis and Epstein-Barr virus infection
- Smoking
- Vitamin D
Low exposure to childhood infections $\rightarrow$ ++ pro-inflammatory immune response $\rightarrow$ High MS risk

Hygiene hypothesis

Leibowitz et al. *J Neurol Neurosurg Psychiatry.* 1966;29:60-68.
Predictions of Hygiene Hypothesis on MS risk

- **Low in developing countries**
- **Increases with SES/education**
- **Increases with Hx of mononucleosis (IM)**

High hygiene ➔ Late EBV infection ➔ IM

P < 0.0000001

Also, Nielsen et al. 2007

Similarities between MS and infectious mononucleosis epidemiology were noted ~ 20 years ago:

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of peak incidence</td>
<td>25-34</td>
<td>15-24</td>
</tr>
<tr>
<td>Age of onset</td>
<td>F&lt;M</td>
<td>F&lt;M</td>
</tr>
<tr>
<td>GEOGRAPHY:</td>
<td></td>
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<tr>
<td>- Extremely rare in the tropics</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>- Latitude gradient within temperate regions</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>- Rare in Japan</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>- Rare in Eskimos</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>Positive association with SES</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Incidence in blacks &lt; whites</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Incidence in Asians &lt; whites</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Sources: adapted from Warner and Carp, Med Hypotheses 1988
Mononucleosis is rare when EBV infection occurs in childhood – becomes common with later age at infection (~ 50%)
% EBV infected at age 4-6 yrs

Barbados
Uganda
Indonesia
Mexico
Hawaii
Brazil
France
Sweden
England
Conn.

% EBV positive

Niederman & Evans, 1997

EBV antibody prevalence at entry at US Military Academy at West Point:

South East
South-West
E.N. Central
South Atl
Mid-Atl
New Engl
North-West

% EBV positive

Hygiene

Hypothesis: high hygiene is a common cause of mononucleosis and MS

No Hygiene

EBV positive child
Low MS risk
Low risk Mononucleosis

EBV negative child
High MS risk
High risk Mononucleosis
Key question: MS risk in EBV negative?

Need sensitive and specific test

EBV -  MS risk high

EBV +
The “EBV paradox” – MS risk in EBV negative individuals:

<table>
<thead>
<tr>
<th>Study</th>
<th>Cases, N</th>
<th>Control Subjects, N</th>
<th>OR of MS for Seronegativity</th>
<th>Exact 95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sumaya and colleagues, 1980</td>
<td>155</td>
<td>76</td>
<td>0.2</td>
<td>0.02–1.24</td>
</tr>
<tr>
<td>2. Bray and colleagues, 1983</td>
<td>309</td>
<td>363</td>
<td>0.11</td>
<td>0.03–0.31</td>
</tr>
<tr>
<td>3. Larsen and colleagues, 1985</td>
<td>93</td>
<td>78</td>
<td>0</td>
<td>0–0.05</td>
</tr>
<tr>
<td>4. Sumaya and colleagues, 1985</td>
<td>104</td>
<td>99</td>
<td>0</td>
<td>0–1.07</td>
</tr>
<tr>
<td>5. Shirodaria and colleagues, 1987</td>
<td>26</td>
<td>24</td>
<td>0</td>
<td>0–5.29</td>
</tr>
<tr>
<td>6. Ferrante and colleagues, 1987</td>
<td>29</td>
<td>31</td>
<td>0.1</td>
<td>0–0.76</td>
</tr>
<tr>
<td>7. Munch and colleagues, 1997</td>
<td>137</td>
<td>124</td>
<td>0.06</td>
<td>0–0.44</td>
</tr>
<tr>
<td>8. Myhr and colleagues, 1998</td>
<td>144</td>
<td>162</td>
<td>0</td>
<td>0–0.67</td>
</tr>
<tr>
<td>9. Wagner and colleagues, 2000</td>
<td>107</td>
<td>153</td>
<td>0</td>
<td>0–0.66</td>
</tr>
<tr>
<td>10. Ascherio and colleagues, 2001</td>
<td>143</td>
<td>269</td>
<td>0.1</td>
<td>0–0.68</td>
</tr>
<tr>
<td>11. Haahr and colleagues, 2004</td>
<td>153</td>
<td>50</td>
<td>0</td>
<td>0–0.82</td>
</tr>
<tr>
<td>12. Sundström and colleagues, 2004</td>
<td>234</td>
<td>693</td>
<td>0</td>
<td>0–1.5</td>
</tr>
<tr>
<td>13. Ponsonby and colleagues, 2005</td>
<td>136</td>
<td>252</td>
<td>0</td>
<td>0–0.96</td>
</tr>
<tr>
<td>Total</td>
<td>1770</td>
<td>2374</td>
<td>152</td>
<td>OR&lt;sub&gt;MH&lt;/sub&gt; = 0.06</td>
</tr>
</tbody>
</table>

*Cornfield confidence interval; *p* < 0.000000001.


OR = odds ratio; MS = multiple sclerosis; CI = confidence interval.

EBV negative individuals have an extremely low risk of MS

HH → IM → MS

EBV and pediatric MS – genetic explanation or other artifacts unlikely

% children with past EBV inf.

OR for EBV NEGATIVE: 0.11; p < 0.001

Source: Alotaibi et al. – JAMA 2004; 292:1875-9

Source: Pohl et al. – Neurology 2006
RR of MS according to EBV infection and history of mononucleosis.

- EBV positive, no history of mononucleosis: Mostly infected with EBV in early childhood
- EBV negative: High hygiene/sanitation, escaped EBV infection in early childhood
- EBV positive, history of mononucleosis: *p<10^-8

RR values:
- 1.0 (Ref)
- 0.06† (p<10^-8)
- 2.3* (p<10^-8)
Over 7 million young adults

= ebv +

= ebv – (~ 3%)

~200,000 EBV – Individuals followed Prospectively x EBV seroconversion and MS incidence
10 cases

MS

EBV +

EBV -

MS

0 cases
Longitudinal study: Time of EBV seroconversion = ▼ and MS onset = ▼

Ascherio et al. Ann Neurol 2010
Conclusions from longitudinal DoDSR study

- EBV infection consistently precedes clinical onset of MS
- Genes cannot explain low MS risk in EBV negative individuals

Ascherio et al. Ann Neurol 2010
Epstein-Barr Virus (EBV)

- Latent infection in B lymphocytes
- Causes B cell proliferation & activation - elicit vigorous and persistent cytotoxic T-cell response
Potential mechanisms linking EBV to MS

Average anti-EBNA complex titers

Cases/Controls  3/46  16/76  38/84  69/136  45/46  46/34

Munger at al. MSJ 2012
**EBV and MS phenotype**

- About 10% of pediatric MS cases appear to be EBV negative.

- It will be interesting to follow these children longitudinally to determine phenotypic differences with EBV + MS.

- In adult MS, data on the relation between EBV Ab titers and MS severity of progression are inconsistent.
Plan of Presentation

- The hygiene hypothesis and Epstein-Barr infection
- Smoking
- Vitamin D
Population: female nurses, U.S.

P, trend = < 0.01

Summary of 4 cohort studies (all women)

- Ascherio & Munger, Ann Neurol 2007
Odds ratio of MS according to smoking history

Source: Riise et al. Neurology 2003
(Table 2). Ever smoking was a significant risk factor for MS in males (OR = 2.8; 95% CI: 1.1–6.9), but not in females (OR = 1.2; 95% CI: 0.68–2.1).

Odds Ratio of MS by pack-years of smoking

Data from Hedstrom et al. Neurology 2009
B. Increase in F:M ratio of cigarette smoking by birth cohort - Canada

Data from: Morozova et al. 2011
Assumptions:
- RR = 1.6 women
- 2.7 in men

Data from: Morozova et al. 2011
Morozova et al. *Annals of Epidemiology*
*Volume 21, Issue 7*, July 2011, Pages 536-542
Figure 1. Kaplan-Meier curve for time to conversion from relapsing-remitting to secondary progressive multiple sclerosis. Smoking status was defined at study entry. Disease in current smokers progressed significantly faster than in never-smokers ($P=0.002$). Red line indicates current smokers; green line, ex-smokers; and black line, never-smokers.
Plan of Presentation

- The hygiene hypothesis and Epstein-Barr infection
- Smoking
- Vitamin D
1. Vitamin D hypothesis:
UV light → higher vitamin D → low MS risk

Limitations of Case – Control studies (not “nested” within cohort)

- Selection bias
- Recall bias
- Reverse causation
- Actinic damage
- Serum 25(OH)
Cohort design

Diet, Sun Exposure

Serum 25(OH)

Low

High

MS

No MS

N.B.: Nested case-control usually equivalent to cohort design
Incident cases of MS = 515
RR of MS according to use of vitamin D supplements

- Vitamin D from supplements, IU/d
  - 0 < 400
  - ≥ 400

- RR of MS
  - 0
  - 0.42
  - 0.68
  - 1.08
  - 0.59
  - 0.38
  - 0.91

- p trend = 0.0006

Nested case-control within the Department of Defense Serum Repository (DoDSR)

DoDSR:
- >40 million serial blood samples since 1990 from over 8 million US military personnel
- Cases (n=257) identified via Physical Disability Agencies
- Controls (n=514) matched by age, sex, race/ethnicity, dates of blood collection
Descriptive characteristics of MS cases and matched controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases n=257</th>
<th>Controls n=514</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>174 (68)</td>
<td>348 (68)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>148 (57.6)</td>
<td>296 (57.6)</td>
</tr>
<tr>
<td>Black</td>
<td>77 (30)</td>
<td>154 (30)</td>
</tr>
<tr>
<td>Hispanic/Other</td>
<td>32 (12.5)</td>
<td>64 (12.5)</td>
</tr>
<tr>
<td>Tier of Residence at Entry, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>North</td>
<td>42 (16.3)</td>
<td>104 (20.2)</td>
</tr>
<tr>
<td>Middle</td>
<td>97 (37.7)</td>
<td>156 (30.4)</td>
</tr>
<tr>
<td>South</td>
<td>101 (39.3)</td>
<td>205 (39.9)</td>
</tr>
<tr>
<td>Outside US</td>
<td>3 (1.2)</td>
<td>6 (1.2)</td>
</tr>
<tr>
<td>Number of serum samples available, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>81 (32)</td>
<td>172 (33.5)</td>
</tr>
<tr>
<td>3</td>
<td>176 (68)</td>
<td>342 (66.5)</td>
</tr>
<tr>
<td>Age First Sample Collected, y mean (sd)</td>
<td>23.3 (5.3)</td>
<td>23.3 (5.3)</td>
</tr>
<tr>
<td>range</td>
<td>16-40</td>
<td>17-41</td>
</tr>
</tbody>
</table>
Rate ratios of MS by quintiles of 25(OH)D adjusted for latitude at entry into the military — Whites

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**Munger et al. JAMA 2006**
RR of MS by tertiles of serum 25(OH)D—Blacks

* Most values < 75 nmol/L

Munger et al. JAMA 2006
Rate ratios of MS by *a priori* category of serum 25(OH)D level—Whites

Munger et al. JAMA 2006
Rate ratios of MS for a 50 nmol/L increase in serum 25(OH)D level by sex —Whites

P for 25(OH)D * sex interaction = 0.90

Munger et al. JAMA 2006
Flow chart of case ascertainment

To identify prospectively collected multiple sclerosis (MS) samples and samples from pregnancies where the offspring had later developed MS, a search for MS and adjacent diagnoses in northern Sweden was performed.

Salzer J et al. Neurology 2012;79:2140-2145
Rate ratios of MS by category of serum 25(OH)D in Swedish cohorts (N = 164,000, 192 MS cases)

Source: Salzer et al. Neurology 2012
Odds of having 25(OH)D levels ≥75 nmol/L according to sampling year time period. Logistic regression was used to calculate odds ratios and confidence intervals for 25(OH)D levels ≥75 nmol/L, by sampling year time periods in 479 good-quality samples from contr...
Effects of different doses of vitamin D on immune responses in human trials

Implications for prevention

- 80% of young adults < 100 nmol/L
- Optimal level (100-150 nmol/L 25(OH)D3) achievable with 1,000 to 4,000 IU supplement/day (considered safe)
- If causal, high potential for MS prevention
- Large randomized trial needed to determine causality – but is this feasible?
Vitamin D and MS treatment

- Growing evidence that:
  - In CIS, low vitamin D levels are associated with faster rate of conversion to MS and higher number of T2 and active lesions.
  - In MS, low vitamin D may be a risk factor for disease progression.
  - Many MS patients have low vitamin D levels: could vitamin D supplements effectively modify disease course?
25(OH)D and relapse rate in 145 patients with MS in Australia

FIGURE 3: Kaplan-Meier survival plots by category of 25-hydroxyvitamin D (25-OH-D) where level of 25-OH-D is determined by the monthly model. The plots show the proportion of subjects relapse-free each day since study entry. Multiple relapses by the same persons are treated as independent observations. The plots and findings were very similar for the as-measured and seasonal models (not shown). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Simpson et al. Ann Neurol 2010
73 patients with RR MS followed for 1.7 years in the Netherlands.

Figure 2: Monthly exacerbation rates for the different groups

(A) Monthly exacerbation rates for the different groups of serum 25-hydroxy-vitamin D (25-OH-D) concentrations, and (B) for at-risk period for infections. Error bars denote 95% confidence intervals. Serum 25-OH-D concentrations: p (trend) = 0.007; infections p < 0.001.

Runia et al. Neurology 2012
FIGURE: Magnetic resonance imaging outcomes associated with quintiles of vitamin D. CI = confidence interval.

Serum 25(OH)D at 6 and 12 months from BENEFIT trial (source: Ectrims 2012)

HR for 50 nmol/L increase in 25(OH)D:

CDMS: 0.40 (0.20-0.77), \( p=0.005 \)

MDMS: 0.30 (0.16-0.56), \( p<0.001 \)
Table 2. HR associated with a *50 nmol/L* increase in 25(OH)D and time to MS conversion by treatment allocation

<table>
<thead>
<tr>
<th></th>
<th>Initial randomization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interferon beta-1b</td>
</tr>
<tr>
<td></td>
<td>HR</td>
</tr>
<tr>
<td>Time to CDMS</td>
<td>0.41</td>
</tr>
<tr>
<td>Time to MDMS</td>
<td>0.39</td>
</tr>
</tbody>
</table>

25(OH)D levels were significantly *inversely* associated with 5-year rate of change in brain volume on MRI
One year double blind placebo-controlled RCT of 20 000 IU/week of vit D3 among 66 RR MS patients on IFNB in Finland

Conclusion: positive effect on MRI outcomes – not powered for clinical outcomes
Other RCT were null, but too small to detect any effect of vitamin D
Possible optimal level for MS prevention, & treatment?

- ≤ 75 nmol/L ("Insufficiency")
- ≤ 50 nmol/L (Deficiency)
- ≤ 25 nmol/L (Severe deficiency)

Ascherio et al. - Vitamin D and MS – Lancet Neurology, 2010
Serum 25(OH)D in MS patients supplemented with high dose vitamin D

SOLAR: ongoing multicenter trial with 14 000 IU/day. 25(OH)D levels expected to raise to over 250 nmol/L: little data on MS at these levels
Although RCT are important, further observational studies may contribute to identify dose-response and possible (genetic?) modifiers.
Acknowledgments:

Harvard:

Kassandra Munger, DSc
Claire Simon, DSc
Eilis O’Reilly, DSc
Natasha Morozova, DSc
Jennifer Massa, MSc
Fariba Mirzaei, MD, MPH
Tanuja Chitnis, MD
Phil De Jager, MD, PhD
Walter Willett, MD, DrPH

Staff: Leslie Unger

Walter Reed Army Institute of Research:
Lynn Levin, PhD

U.S. Army and Navy Participants in NHS cohorts
BENEFIT participants & Investigators
Prof. Ludwig Kappos
Dr. Christoph Pohl