Month of Birth and Thymic Output

It is widely acknowledged that month of birth influences the risk for developing multiple sclerosis (MS) later in life. This has been consistently shown in a number of countries and regions including England, Scotland, Canada, Denmark, Sweden, Sardinia, Finland, and Australia (eReferences, http://www.jamaneurop.com). The month of birth effect is particularly evident in England, where the risk for MS peaks in May and drops in November for those born in those months. The biological basis of this month of birth effect is not yet known. Because thymic development mainly occurs in utero and is susceptible to intrauterine exposures, we sought to assess whether the month of birth has any effect on thymic function. Given the evidence accumulating for vitamin D deficiency in the etiology of MS, we also investigated whether vitamin D levels were associated with thymic output.

Methods. The basic mechanism for generating T-cell receptors (TCRs) during T-cell development provides an assay to measure thymic output. Signal sequences flanking the TCR gene segments in their germline configuration are cleaved during TCR production and join to form extrachromosomal DNA circles termed signal joint TCR excision circles (sjTRECs). Cord blood was obtained from 50 healthy white individuals born in November and 50 born in May between 2009 and 2010 in London after ethical approval. CD4+ and CD8+ T-cell populations were separated using magnetic microbeads. Quantification of sjTRECs in CD4+ and CD8+ populations was performed by real-time quantitative polymerase chain reaction. Samples were analyzed in duplicate. Concentrations of 25-hydroxyvitamin D were measured by isotope-dilution liquid chromatography–tandem mass spectrometry.

Results. We observed a significantly greater number of sjTRECs in CD4+ and CD8+ cells from individuals born in May compared with those born in November (P = 5.5 × 10^-3 and P = 1.2 × 10^-6, respectively; Table). Individuals born in May had significantly less circulating 25-hydroxyvitamin D than those born in November (P = .02), and there was also an inverse correlation between 25-hydroxyvitamin D and CD4+ and CD8+ sjTRECs (Spearman rho = -0.37, P = .009; and Spearman rho = -0.4, P = .004, respectively).

Comment. By looking at TRECs, we observed that month of birth has a significant effect on thymic output. We hypothesized that birth month influences T-cell production and may impair T-cell central tolerance and/or T-regulatory/T-effector cell balance, predisposing to MS. These findings are particularly interesting given the recent observation that MS-associated genes are active in cord blood T cells. Taken together, these data highlight how genetic and environmental risk factors act in the same cell types and interact in the etiology of MS. Interestingly, the risk for immune conditions other than MS, including type 1 diabetes mellitus, rheumatoid arthritis, and ulcerative colitis, is also higher in spring-born individuals, suggesting the presence of shared etiologic pathways across these disorders.

Vitamin D may be the driver of this effect. Notably, genetic regions associated with immune-mediated diseases, including MS, are significantly enriched for vitamin D–receptor binding sites, and vitamin D is able to regulate the expression of hundreds of genes, many of which play roles in T-cell and B-cell development. Animal studies have shown that in utero vitamin D deficiency has a significant effect on the developing immune system. To our knowledge, our findings represent the first direct evidence suggesting that the same happens in the developing human immune system. In summary, our data supports a role for vitamin D underlying the month of birth effect in MS through effects on thymic development and T-cell production. Long-term prospective studies should be initiated to investigate the effect of gestational vitamin D supplementation on thymic output and, ultimately, the subsequent risk for MS.

Table. CD4+ and CD8+ sjTRECs and 25-hydroxyvitamin D Among Individuals Born in May and November

<table>
<thead>
<tr>
<th></th>
<th>sjTRECs/100 000 Cells, Mean (95% CI)</th>
<th>Monthly 25-hydroxyvitamin D, Mean, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>November</td>
<td>CD4+ 11 089 (10 205-11 973)</td>
<td>50.9</td>
</tr>
<tr>
<td></td>
<td>CD8+ 10 414 (9 509-11 319)</td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>CD4+ 19 547 (17 609-21 485)</td>
<td>38.4</td>
</tr>
<tr>
<td></td>
<td>CD8+ 25 520 (22 488-28 552)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: sjTRECs, signal joint T-cell receptor excision circles.

Giulio Disanto, MD
Corey T. Watson, PhD
Ute C. Meier, DPhil
George C. Ebers, MD
Gavin Giovanni, MD
Sreeram V. Ramagopalan, DPhil

Author Affiliations: Department of Physiology, Anatomy, and Genetics, and Medical Research Council Functional Genomics Unit, University of Oxford, Oxford (Drs...
Disanto, Watson, Ebers, and Ramagopalan); Blizard Institute, Queen Mary University of London, Barts and The London School of Medicine and Dentistry, London (Drs Meier, Giovannoni, and Ramagopalan), England.

Correspondence: Dr Ramagopalan, Department of Physiology, Anatomy, and Genetics, and Medical Research Council Functional Genomics Unit, University of Oxford, South Parks Rd, Oxford, OX1 3PT, England (sreeram.ramagopalan@dpag.ox.ac.uk).

Author Contributions: Drs Disanto and Ramagopalan had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Meier, Ebers, Giovannoni, and Ramagopalan. Acquisition of data: Watson and Ramagopalan. Analysis and interpretation of data: Disanto, Watson, Ebers, Giovannoni, and Ramagopalan. Drafting of the manuscript: Disanto and Ramagopalan. Critical revision of the manuscript for important intellectual content: Watson, Meier, Ebers, and Giovannoni. Statistical analysis: Ramagopalan. Obtained funding: Ebers, Giovannoni, and Ramagopalan. Administrative, technical, and material support: Ramagopalan. Study supervision: Meier, Ebers, and Ramagopalan.

Conflict of Interest Disclosures: Dr Ebers serves on the editorial boards of the International Multiple Sclerosis Journal and Multiple Sclerosis and as section editor for BMC Medical Genetics; has received funding for travel or speaker honoraria from Bayer Schering Pharma, Sanofi-Aventis, Roche, and UCB; and has served as a consultant to Biopartners, Bayer Schering Pharma, Howrey LLP, Heron Health, and Eli Lilly and Co. Dr Ebers receives research support from Bayer Schering Pharma, the Multiple Sclerosis Society of the United Kingdom, and the Multiple Sclerosis Society of Canada Scientific Research Foundation. Dr Giovannoni serves on scientific advisory boards for Merck Serono, Biogen Idec, and Vertex Pharmaceuticals; has served on the editorial board of Multiple Sclerosis; has received speaker honoraria from Bayer Schering Pharma, Merck Serono, Biogen Idec, Pfizer Inc, Teva Pharmaceutical Industries Ltd, Sanofi-Aventis, Vertex Pharmaceuticals, Genzyme Corp, Ironwood, and Novartis; has served as a consultant for Bayer Schering Pharma, Biogen Idec, GlaxoSmithKline, Merck Serono, Protein Discovery Laboratories, Teva Pharmaceutical Industries Ltd, Sanofi-Aventis, UCB, Vertex Pharmaceuticals, GW Pharma, Novartis, and FivePrime; and serves on the speakers bureau for Merck Serono. Dr Giovannoni has also received research support from Bayer Schering Pharma, Biogen Idec, Merck Serono, Novartis, UCB, Merz Pharmaceuticals LLC, Teva Pharmaceutical Industries Ltd, Sanofi-Aventis, GW Pharma, and Ironwood.

Funding/Support: This work was funded by grant G0801976 from the Medical Research Council and a research fellowship from the Fondazione Italiana Sclerosi Multipla (cod 2010/B/5).

Role of the Sponsors: The study sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.