Comment on "The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease"

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Comment on “The Influence of the Proinflammatory Cytokine, Osteopontin, on Autoimmune Demyelinating Disease”

Osteopontin (OPN), also called early T cell activation gene-1 (Eta-1) or secreted phosphoprotein 1 (Spp1), has important functions in bone metabolism (1, 2) and in inflammation and immunity to infectious diseases (3, 4). Recently, several studies (5–9) have suggested that OPN also plays a crucial role in inflammatory disease models of multiple sclerosis (MS) and rheumatoid arthritis (RA). The studies concluded that OPN also plays a crucial role in inflammatory disease models of MS (6) and RA (7). In most cases, the effects of OPN deletion were consistent, regardless of the genetic background of the mice, although there were some differences. For example, in the MS model, deletion of OPN resulted in a reduced severity of disease, while in the RA model, deletion of OPN resulted in a reduced severity of disease.

The flaw in these data is that they may be explained by linked polymorphic genes, because the mice were not fully backcrossed and typed in these experiments. We have deleted the OPN gene using homologous recombination of strain 129-derived cells, and have subsequently backcrossed it to the C57BL10 strain with a congenic major histocompatibility complex (MHC) fragment of the q haplotype (B10.Q), which is usually susceptible to EAE, collagen induced arthritis (CIA), and anti-CII antibody transfer induced arthritis (CAIA) (10–12). The gene was shown to be completely inactivated, with no aberrant transcript. The mice were backcrossed for 12 generations to the C57/Black background, and the remaining linked fragment was determined with microsatellite marker between positions 45 and 64 centiMorgan (cM) on chromosome 5. In all experiments, both wild-type B10.Q littermates and heterozygous littermates were used as controls.

In contrast to the findings published by Chabas et al. (5) and others, we saw no effect on any inflammatory model tested—EAE, CIA, or CAIA (Table 1). In our EAE experiments we used 25 μg recombinant rat myelin oligodendrocyte glycoprotein (MOG) emulsified in complete Freund’s adjuvant (CFA) using a 0-to-8 scoring scale, as described in detail in (10). The mice were followed for 37 days, and 20% of them were in remission at the end of the experiment. A direct comparison with previously published experiments shows that our experimental duration covered the same periods during which the severity differences between the groups were highly significant in the experiments by Chabas et al. (day 35 (5)), as well as in experiments by Jansson et al. (days 14 to 15 (9)). The wild-type controls in all experiments were of similar severity, but we found no significant difference for our OPN-deleted mice in severity or in remissions at any time point. Possibly, the MOG-induced disease could be different from the MOG peptide-induced disease or proteolipid protein peptide (PLP)–induced disease used in the model of Chabas et al. (5), but that would severely limit the transferability of the data from the Chabas et al. experiments to actual MS. Another possible explanation is that the OPN-deficient mouse used by others still has an aberrant transcript (13), whereas OPN is completely deleted from our model. That difference, however, is unlikely to explain the observed discrepancy. The OPN+/−129/B6 mice also had a dramatic reduction of acute inflammation induced by monoclonal anti-type II collagen antibodies compared with OPN wt 129/B6 in experiments by Yumoto et al. (8), which used a model very similar to the arthritis induced in our mice (12). This is important, because this model is an acute inflammation but is not dependent on T or B cells.

It is more likely that the observations of Chabas et al. (5) and others (6–9) resulted from one or several polymorphic genes derived from strain 129 and linked to the OPN locus. In these experiments—in which mixed or incompletely backcrossed 129/B6 mice containing homozygous OPN or homozygous “wild type” were used—a large and unidentified fragment on chromosome 5, derived from 129 and containing a deleted OPN gene, was compared with a corresponding fragment from B6. Thus, any polymorphic genes in this fragment, which contains thousands of genes, could be of importance. Indeed, several quantitative trait loci (QTLs) have been observed on chromosome 5 in linkage studies of Lyme arthritis (14) and EAE (15). In addition, a gene within an earlier-defined QTL in a homologous region in rats has recently been cloned that dramatically affects the severity of arthritis and EAE (16). This region has also long been known to control traits like resistance to Rickettsia infection, and the polymorphism of the associated haplotype has been found to vary extensively among strains (17). Thus, this region—as is probable with most parts of the genome—contains many polymorphic genes that can potentially have an important influence on inflammatory diseases. A list of some closely linked polymorphic genes that potentially could influence an inflammatory response is provided in Table 2.

There are several difficulties in using knockout mice in providing conclusive evidence for genes in disease traits, like the role of OPN in inflammatory diseases. A common pitfall is when wild-type mice and non-littermates are used as controls: In that situation, any gene in the genome that differs between the embryonic stem (ES) cell and the backcrossed parental cell may play a role. Preferably, heterozygous littermates should be included, because dominant genes in the linked fragment will be neutralized. However, a recessive effect closely linked to the gene of interest is almost impossible to exclude if the knockout is created with ES cells of a different genotype than in the mouse used later in the study. In addition, a parental strain 129 control is not proper as a control for many different reasons. The origins of 129 strains vary and are most likely different from those of the abnormal strain that grows from a somatically mutated ES cell line that has been

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**Table 1. Severity, incidence, and onset in CIA, EAE, and CAIA. wt, wild-type.**

<table>
<thead>
<tr>
<th>Model</th>
<th>Genotype</th>
<th>Mean maximum score (±SD)</th>
<th>Incidence, % (numbers)</th>
<th>Mean onset day (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIA</td>
<td>wt/wt</td>
<td>20.8 (±7.2)</td>
<td>54.5 (6/10)</td>
<td>44.5 (±6.2)</td>
</tr>
<tr>
<td></td>
<td>opn/-opn-</td>
<td>23.7 (±5.8)</td>
<td>50.0 (3/6)</td>
<td>47.3 (±9.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.8 (±2.9)</td>
<td>50.0 (4/8)</td>
<td>41.3 (±6.8)</td>
</tr>
<tr>
<td>EAE</td>
<td>opn/-opn-</td>
<td>6.1 (±0.2)</td>
<td>91.7 (11/12)</td>
<td>13.2 (±0.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.3 (±0.2)</td>
<td>100 (10/10)</td>
<td>12.8 (±0.6)</td>
</tr>
<tr>
<td>CAIA</td>
<td>opn/-opn-</td>
<td>3 (±0)</td>
<td>25 (1/4)</td>
<td>3 (±0)</td>
</tr>
<tr>
<td></td>
<td>7 (±1.1)</td>
<td>36 (3/10)</td>
<td>7.33 (±1.2)</td>
<td></td>
</tr>
</tbody>
</table>
adapted for growth in a laboratory. Furthermore, it is well known that isolated congenic fragments will control traits different from their role in the parents because of interactions with the rest of the genome, as well as through splitting of selected gene pairs in the borders of the congenic fragments. Therefore, it is of general and crucial importance to identify the naturally selected polymorphisms controlling the diseases or traits of interest.

We emphasize that we do not question an important and potential role of OPN in various biological contexts (1, 2). The role for OPN in inflammatory disease is still an open issue. The lack of effect in the OPN-deleted mouse is most likely explained by the influence of other genes that may replace the role of OPN. Identifying the OPN-linked polymorphic genes that exert a strong influence on arthritis and encephalomyelitis therefore represents a challenging and important task.

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Table 2. Genes closely linked to OPN (Spp1) that have inflammatory functions and their position along chromosome 5 (in cM).

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Name</th>
<th>Position</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cxcl1</td>
<td>Chemokine (C-X-C) ligand 1</td>
<td>51</td>
<td>Chemotactant, APC activation</td>
<td>(18, 19)</td>
</tr>
<tr>
<td>Cxcl2</td>
<td>Chemokine (C-X-C) ligand 2</td>
<td>51</td>
<td>Chemotactant, neutrophil Ca2+ release/activation</td>
<td>(20, 21)</td>
</tr>
<tr>
<td>Cxcl5</td>
<td>Chemokine (C-X-C) ligand 5</td>
<td>51.5</td>
<td>Chemotactant, hemotopoiesis</td>
<td>(22)</td>
</tr>
<tr>
<td>Cxcl9</td>
<td>Chemokine (C-X-C) ligand 9</td>
<td>53</td>
<td>Chemotactant, anti-angiogenesis</td>
<td>(21, 23)</td>
</tr>
<tr>
<td>Cxcl10</td>
<td>Chemokine (C-X-C) ligand 10</td>
<td>53</td>
<td>Chemotactant, anti-angiogenesis</td>
<td>(24, 25)</td>
</tr>
<tr>
<td>Bmp3</td>
<td>Bone morphogenetic protein 3</td>
<td>55</td>
<td>Bone metabolism, TGF beta signalling</td>
<td>(24, 26)</td>
</tr>
<tr>
<td>Fgfl</td>
<td>Fibroblast growth factor 5</td>
<td>55</td>
<td>Fibroblast proliferation, brain development</td>
<td>(27, 28)</td>
</tr>
<tr>
<td>Sparc1</td>
<td>SPARC-like 1</td>
<td>55</td>
<td>B cell development</td>
<td>(29, 30)</td>
</tr>
<tr>
<td>*Spp1</td>
<td>Secreted phosphoprotein 1 (OPN)</td>
<td>56</td>
<td>Thymus size (polymorphic between C56/BL6 and C56/BL10)</td>
<td>(31, 32)</td>
</tr>
<tr>
<td>Tsz1</td>
<td>Thymus size 1</td>
<td>56</td>
<td>Leucocyte tethering/rolling</td>
<td>(33)</td>
</tr>
<tr>
<td>Selpl</td>
<td>Platelet selectin ligand</td>
<td>64</td>
<td>Thymus size (polymorphic between C56/BL6 and C56/BL10)</td>
<td>(34, 35)</td>
</tr>
<tr>
<td>Lnk</td>
<td>Linker of T cell pathways</td>
<td>65</td>
<td>Cytokine signaling and hematopoietic homeostasis</td>
<td>(36, 37)</td>
</tr>
<tr>
<td>Nos1</td>
<td>Nitric oxide synthase 1, neuronal</td>
<td>65</td>
<td>Inflammation, asthma</td>
<td>(38, 39)</td>
</tr>
</tbody>
</table>