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

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Published in:
Science

DOI:
10.1126/science.1078985

2003

TECHNICAL COMMENTS

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). Chabas *et al*. (*5*) first suggested OPN involvement in MS, based on both expression analysis data from MS-affected brains and from studies of mice with the OPN gene deleted; those mice were shown to be partly protected from experimental autoimmune encephalomyelitis (EAE). This was followed up by several papers using other models for RA and MS (*8, 9*).

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 C57/BL10 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 contained 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 not 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

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

Model	Genotype	Mean maximum score (±SD)	Incidence, % (numbers)	Mean onset day (± SD)
CIA	wt/wt	20.8 (±7.2)	54.5 (6/10)	44.5 (±6.2)
	opn-/wt	23.7 (±5.8)	50.0 (3/6)	47.3 (±9.3)
	opn-/opn-	23.8 (±2.9)	50.0 (4/8)	41.3 (±6.8)
EAE	opn-/wt	6.1 (±0.2)	91.7 (11/12)	13.2 (±0.9)
	opn-/opn-	6.3 (±0.2)	100 (10/10)	12.8 (±0.6)
CAIA	opn-/wt	3 (±0)	25 (1/4)	3 (±0)
	opn-/opn-	7 (±1.1)	36 (3/10)	7.33 (±1.2)

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

Symbol	Name	Position	Function	Reference
Cxcl1	Chemokine (C-X-C) ligand 1	51	Chemoattractant, APC activation	(*18, 19*)
Cxcl2	Chemokine (C-X-C) ligand 2	51	Chemoattractant, neutrophil Ca^{2+} release/activation	(*20, 21*)
Cxcl15	Chemokine (C-X-C) ligand 15	51.5	Chemoattractant, hematopoiesis	(*22*)
Cxcl5	Chemokine (C-X-C) ligand 5	53	Chemoattractant, neutrophil Ca^{2+} release/activation	(*21, 23*)
Cxcl9	Chemokine (C-X-C) ligand 9	53	Chemoattractant, anti-angiogenesis	(*24, 25*)
Cxcl10	Chemokine (C-X-C) ligand 10	53	Chemoattractant, anti-angiogenesis	(*24, 26*)
Bmp3	Bone morphogenic protein 3	55	Bone metabolism, TGF beta signalling	(*27, 28*)
Fgf5	Fibroblast growth factor 5	55	Fibroblast proliferation, brain development	(*29, 30*)
Sparcl1	SPARC-like 1	55	B cell development	(*31, 32*)
*Spp1	Secreted phosphoprotein 1 (OPN)	56		
Tsz1	Thymus size 1	56	Thymus size (polymorphic between C56/BL6 and C56/BL10)	(*33*)
Selpl	Platelet selectin ligand	64	Leucocyte tethering/rolling	(*34, 35*)
Lnk	Linker of T cell pathways	65	Cytokine signaling and hematopoietic homeostasis	(*36, 37*)
Nos1	Nitric oxide synthase 1, neuronal	65	Inflammation, asthma	(*38, 39*)

adapted for growth in a laboratory. Furthermore, it is well known that isolated congenic fragments will control traits different from their role in the parentals 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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30 September 2002; accepted 31 December 2002