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Regulation of T-helper-cell lineage development by osteopontin:

the inside story

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Abstract

Studies of osteopontin (OPN)-dependent regulation of immune responses have focused on the cytokine activities of the secreted form of this protein. Recent evidence has revealed that an intracellular form of OPN expressed by dendritic cells regulates the expression of pro-inflammatory cytokines and the differentiation of T helper (T_H)-cell lineages. In this Opinion article, we discuss the properties of both OPN isoforms and their respective contributions to the immune response. We propose that cell-type-specific expression of secreted and intracellular OPN regulates the development of distinct effector T_H cells, including that of T_H1 and T_H17 cells.

Osteopontin (OPN; also known as ETA1 and encoded by *SPPI*) has been recognized as a T-bet-dependent pro-inflammatory cytokine that is produced by activated T helper 1 (T_H1) cells. In dendritic cells (DCs), OPN-dependent ligation of $\alpha\text{v}\beta 3$ integrin induces the expression of interleukin-12 (IL-12), whereas engagement of CD44 by OPN inhibits the expression of IL-10 (REFS 1²). The interactions of OPN with CD44 and $\alpha\text{v}\beta 3$ integrin also promote the chemotactic migration of DCs and Langerhans cells³. Early studies that examined the contribution of OPN to immune responses focused almost exclusively on the activities of the secreted isoform of OPN (sOPN). More recently, studies have revealed that antigen-presenting cells (APCs), including macrophages and DCs, also express an intracellular form of OPN (iOPN), which regulates cell motility, cell division and cytokine expression^{4–8}. Molecular analysis of the expression of the *Opn* gene by DCs has shown that alternative translation of a single species of *Opn* mRNA generates two protein isoforms (sOPN and iOPN)⁸ that regulate the development of T_H-cell subsets^{1,2,9–11}. In this Opinion article, we propose that the regulation of the T_H1- and T_H17-cell responses through the differential expression of sOPN and iOPN by immune cells determines the type and intensity of adaptive immune responses to foreign and self antigens.

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DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
 $\alpha\text{v}\beta 3$ [CD44][IFN α][IFN γ][IL-10][IL-12][IL-27][IRF7][MyD88][OPN][TLR9]

OMIM: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>
multiple sclerosis[rheumatoid arthritis]systemic lupus erythematosus

FURTHER INFORMATION

Harvey cantor's homepage: <http://www.hms.harvard.edu/dms/immunology/fac/cantor.html>

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OPN and antimicrobial responses

The contribution of OPN to protective immunity against microbial infection was first suggested by findings that the *Opn* gene mapped to a locus (*Ric*) on mouse chromosome 5 that confers resistance to infection by *Rickettsia tsutsugamushi*, an obligate intracellular bacterium that causes human scrub typhus^{12,13}. Subsequent studies suggested that OPN expression also contributes to protective immunity against several additional intracellular pathogens, including viruses, bacteria and protozoa¹⁴⁻¹⁹.

Further evidence of the contribution of OPN to the protection against intracellular pathogens came from a study on OPN-deficient mice, which were more susceptible to infection with *Mycobacterium bovis* than OPN-sufficient mice¹⁵. Although the expression of sOPN may also promote antimicrobial immunity through the suppression of the anti-inflammatory cytokine IL-10 (REF. 1), control of mycobacterial infection depends mainly on the induction of an IL-12 response²⁰. Moreover, analysis of plasma from patients with tuberculosis revealed that high levels of sOPN correlated with increased levels of IL-12 (REFS 17,18), perhaps reflecting an interaction between sOPN and its receptor $\alpha\beta 3$ integrin, which can upregulate IL-12 secretion by APCs¹⁴. OPN expression (detected by immunohistochemistry) in patients with tuberculosis was also associated with efficient granuloma formation and inversely correlated with mycobacterial dissemination and disease mortality¹⁶.

Analysis of OPN-deficient mice suggested that this protein also contributes to protection against other intracellular pathogens, including rotavirus¹⁹, herpes simplex virus type 1 (REFS 1,9), *Listeria monocytogenes*¹ and *Plasmodium falciparum*¹⁰. Although protection against intracellular pathogens has generally been attributed to the expression of sOPN, protection against some of these microorganisms may also be the result of iOPN expression. Several studies using immunohistochemistry or flow cytometry showed that OPN was present in pathogen-infected cells^{10,16}. However, additional investigation is required to determine whether these studies reflect the presence of iOPN, sOPN or both isoforms.

The protective role of OPN against infection does not extend to all types of pathogen. For example, OPN deficiency does not attenuate resistance to some viruses, including influenza virus²¹, or to the extracellular bacterium *Borrelia burgdorferi* in mice²². In the study examining the effect of OPN deficiency on the protection against viral infections²¹, OPN-independent production of antiviral cytokines, including interferon- α (IFN α), could have bypassed the need for OPN-dependent protection²³. In the case of *B. burgdorferi* infection²², the pathogen inocula that were used in this study might have been insufficient to induce OPN-dependent protection, as suppression of IL-10 by OPN, which results in enhanced resistance to *B. burgdorferi*²⁴, may require higher titres of this bacterium.

In addition to sOPN-dependent induction of the pro-inflammatory cytokine IL-12 (REF. 1), iOPN expression by plasmacytoid DCs (pDCs) can enhance T_H1-cell responses through the upregulation of IFN α expression by these cells following viral infection⁹. Recent studies also showed that iOPN expression by conventional DCs (cDCs) induces the development of T_H17 cells¹¹ (see below). However, studies on T_H17-cell protective immune responses that are mediated by OPN have not yet been conducted.

OPN and autoimmune diseases

The relationship between OPN expression and autoimmune disease was first suggested by findings showing that overexpression of *Opn* mRNA positively correlated with the onset and increased severity of systemic lupus erythematosus (SLE) in mice²⁵. In addition, *Opn* allele-specific effects were observed for development of the SLE-like disease in susceptible mouse strains: the presence of *Opn*^{MAL} predisposes animals to lupus-associated nephritis, whereas the

presence of *Opn*^{C3H} does not²⁶. High levels of OPN have also been reported in patients with autoimmune lymphoproliferative syndrome²⁷, and polymorphisms of the human *OPN* locus have been linked to susceptibility to SLE^{28,29}.

Furthermore, OPN is thought to contribute to the pathogenesis of some forms of arthritis, as OPN-deficient mice are protected from joint destruction that occurs during collagen-antibody-induced arthritis³⁰. In addition, the pathogenesis of collagen-antibody-induced arthritis and collagen-induced arthritis can be ameliorated by binding of the OPN antibody to a cryptic epitope that is exposed on sOPN after it is cleaved by thrombin^{31,32}. By contrast, OPN-deficient mice were not resistant to arthritis in other studies that used a different model of collagen-induced arthritis or in the K/BxN arthritis models^{33,34}. Although the reason for these discrepant results is currently unknown, these findings emphasize the distinct pathogenic pathways that are responsible for the development of arthritis in different mouse models.

OPN has been implicated in the development of autoimmune diseases that are mediated by T_H1 or T_H17 cells. However, sOPN has also been implicated in allergic airway diseases, which are mediated by T_H2 cells³⁵. Inhibition of pDC migration to draining lymph nodes by sOPN resulted in enhanced pro-inflammatory responses and airway hypersensitivity during primary systemic sensitization. In addition, sOPN-dependent inhibition of the migration of cDCs to pulmonary lymph nodes following allergen challenge suppressed the pulmonary allergic response³⁵. Thus, sOPN regulates T_H2-type responses in airway hypersensitivity through temporal inhibition of either pDC or cDC migration. How sOPN achieves temporally distinct inhibition of pDC and cDC migration is not understood. It is possible that sOPN-dependent retention of recently arrived cDCs in draining lymph nodes through the suppression of CC-chemokine receptor 5 (CCR5) and CCR7 expression may prevent excessive homing of activated cDCs to inflamed tissues and draining lymph nodes³⁶. Further studies are necessary to fully delineate the role of sOPN (and iOPN) in T_H2-type responses.

Intracellular OPN

Translation of sOPN is initiated from the AUG codon that is located at the 5'-end of the *Opn* signal sequence, which targets full-length nascent sOPN to secretory vesicles⁸ (FIG. 1). Evidence for an intracellular form of OPN (iOPN) that localizes outside of secretory vesicles was initially inferred from its perinuclear distribution in rat calvarial cells⁴. iOPN is a shorter isoform of sOPN that is produced by alternative translation, which is initiated downstream of the conventional AUG start site and therefore lacks the signal sequence at the amino terminus. Expression of the two isoforms differs depending on cell type: DCs express high levels of iOPN but low levels of sOPN, whereas the reverse is true of activated T cells². It is possible that cell-type-specific factors may regulate the alternative translation of *Opn* mRNA. Analysis of the subcellular localization of iOPN in DCs by confocal microscopy and gradient separation has confirmed that it is localized in the cytoplasm and dendrites⁸, but not in the Golgi apparatus. This expression pattern reflects the direct entry of nascent iOPN protein into the cytoplasm following its synthesis⁸.

The functional properties of iOPN suggest that it may function as an adaptor protein rather than as a catalytic molecule. It colocalizes with myeloid differentiation primary-response gene 88 (MyD88) and Toll-like receptor 9 (TLR9) in DCs, and with ezrin (a component of the ezrin-radixin-moesin complex), polo-like kinase 1 and the cytoplasmic domain of CD44 in other cell types^{5-7,9,37}. iOPN is a member of the SIBLING (small integrin-binding ligand N-linked glycoproteins) family, the members of which display extended folding in solution and have structural features that enable them to interact with multiple binding partners and to facilitate the organization of specialized macromolecular clusters³⁸.

iOPN in pDCs and T_H1-cell responses

Although all nucleated cells can produce type I IFNs, pDCs are specialized cells that rapidly secrete high levels of type I IFNs following viral stimulation. binding of type I IFNs to the type I IFN receptor (IFNAR)³⁹ upregulates IFN γ production; therefore, this pathway favours the induction and maintenance of T_H1-cell responses⁴⁰⁻⁴².

Engagement of TLR9 results in T-bet-dependent expression of iOPN in pDCs⁹. Association of nascent iOPN with MyD88 results in the activation of the transcription factor IFN-regulatory factor 7 (IRF7) and induction of *Ifna* expression⁹. Although TLR9-dependent activation of MyD88 can also activate the transcription factor nuclear factor- κ B (NF- κ B) and induce the expression of pro-inflammatory cytokines, including IL-6 and tumour-necrosis factor (TNF), iOPN has been shown to selectively couple TLR9 activation with that of IRF7 instead of NF- κ B⁹.

Some of the functional consequences of this iOPN-dependent pathway for IFN α production in pDCs have been delineated. iOPN expression by pDCs is essential for IFN α -dependent antigen cross-presentation to CD8⁺ T cells, resistance to infection by herpes simplex virus type 1 and induction of natural-killer cell cytotoxicity that protects mice from the lethal effects of B16 melanoma⁹. These findings are consistent with the view that iOPN expression by pDCs is essential for IFN α -dependent immunity and T_H1-cell responses *in vivo*.

iOPN in cDCs and T_H17-cell responses

T_H1 and T_H2 cells enhance the clearance of intracellular bacteria and viruses or parasites, respectively. A recently defined third subset of T_H cells that produce IL-17 — termed T_H17 cells — can protect the host from infection by extracellular bacteria and fungi, including clinically important pathogens such as *B. burgdorferi* and *Candida albicans*^{43,44}. However, IL-17 can also promote tissue damage, and uncontrolled T_H17-cell responses are thought to contribute to several human autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, Crohn's disease, ulcerative colitis and SLE⁴⁵⁻⁵⁰.

Recent studies have shown that iOPN expression by cDCs has a key role in promoting T_H17-cell responses. iOPN suppresses the expression of IL-27, which is known to inhibit the development of T_H17 cells¹¹. In addition, OPN-deficient cDCs secrete high levels of IL-27 and therefore attenuate the T_H17-cell response. This suggests that iOPN releases the 'brake' on the T_H17-cell responses by suppressing IL-27 (REFS 51,52). Whether sOPN expression by activated T cells also participates in the generation or maintenance of the T_H17-cell response is currently being investigated.

Furthermore, engagement of IFNAR has been shown to suppress the expression of iOPN, which leads to enhanced secretion of IL-27 and, consequently, to attenuated T_H17-cell responses^{11,53}. In addition, engagement of IFNAR following viral infection results in an IL-27-dependent blockade of T_H17-cell responses¹¹. This effect may prevent unwanted or excessive inflammatory responses during viral infection, without compromising the protective antiviral immunity that is mediated by pDCs and T_H1 cells. T_H17-cell responses may be further inhibited by the increased iOPN-dependent secretion of type I IFNs by pDCs following TLR7 or TLR9 activation by viral RNA or DNA, which would activate IFNAR on the surface of cDCs (FIG. 2). Therefore, this interaction between the two main DC subsets may regulate the level of activation of T_H-cell subsets.

OPN in the central nervous system

The identification of a potential role for OPN in the pathogenesis of multiple sclerosis came from findings that plaques from the brain and spinal cords of rats with experimental

autoimmune encephalomyelitis (EAE) contained high levels of *Opn* transcripts⁵⁴ and that OPN-deficient mice showed decreased development of the disease^{54,55}. The initial concern that other genes linked to the *Opn* locus might be responsible for the second finding proved to be unfounded⁵⁶, as administration of recombinant OPN to wild-type mice exacerbated disease⁵⁷, and the attenuated phenotype that was observed in OPN-deficient mice was still apparent after 16 generations of backcrossing².

Both sOPN and iOPN have been shown to regulate EAE development. T-cell-derived sOPN can inhibit apoptosis of pathogenic T cells, thereby sustaining T-cell pathogenicity during the relapse and progression of EAE⁵⁷. In addition, the expression of iOPN by APCs, rather than T cells, regulates the onset of EAE¹¹ and the generation of proinflammatory T_H17 cells, as determined by immunization with antigen-pulsed DCs that differ in their expression of iOPN¹¹. These findings suggest that iOPN expression by DCs exacerbates EAE development by promoting the T_H17-cell response, whereas sOPN may sustain disease by dampening apoptosis of pathogenic T cells (FIG. 3).

Future prospects

Although IFN β therapy is currently the leading treatment for multiple sclerosis⁵⁸, the underlying mechanism of its effect on disease progression is not understood. Recent studies^{11,53} suggest that suppression of the T_H17-cell response by the IFNAR-dependent pathway described above (FIG. 2) could account for its ameliorative effects through two pathways. IFNAR signalling induces IL-27 production and thereby suppresses T_H17-cell responses and ameliorates EAE^{11,53}. Moreover, engagement of IFNAR expressed by myeloid cells that reside in the central nervous system can inhibit cytokine and chemokine production and diminish phagocytosis of myelin by these cells³⁹.

These data indicate that it may be possible to produce new IFNAR agonists that more efficiently activate the iOPN-IL-27 pathway and thus diminish the T_H17-cell response without activating antiviral pathways that may lead to IFN β -related side effects. This approach has already been initiated using a 'mutate and screen' method⁵⁹, which has successfully isolated mutant IFN α molecules that induce increased antiviral and T_H1-cell responses without concomitant increases in anti-proliferative potential⁶⁰. Although the study did not evaluate the effect of these mutant IFN α molecules on T_H17-cell responses, it is plausible that the responses may be altered by interactions between IFNAR and IFN α ligands that contain appropriate mutations. Increased suppression of iOPN by IFNAR agonists may also serve as an important marker for the evaluation of new treatments for multiple sclerosis.

Research into the contributions of OPN to the regulation of immune responses has focused primarily on the cytokine-like activities of sOPN that is produced by T cells. However, recent studies have uncovered a new isoform of OPN that is expressed as an intracellular protein in DCs and macrophages. Analysis of the contribution of iOPN expression to the immunological activities of DCs has suggested a key role for this protein in DC-dependent regulation of T_H1- and T_H17-cell responses. In view of recent studies suggesting that both T_H1 and T_H17 cells are involved in the pathogenesis of multiple sclerosis and EAE (reviewed in REF. 61), the ability of OPN isoforms to regulate T_H1- and T_H17-cell responses may prove to be relevant to new types of treatment for clinically distinct subsets of multiple sclerosis. The task ahead is to further define the individual and combined contribution of the two OPN isoforms to the development of the different T_H-cell subsets in the context of protective immunity and autoimmune disease. This can be achieved, in part, by analysing cells or animals that are genetically engineered to express sOPN or iOPN in different cell types and by the translation of these findings to new approaches that regulate the expression of the two OPN isoforms in different diseases.

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Figure 1. Alternative translational mechanism that generates sOPN and iOPN

Following transcription and processing, mature osteopontin (*OPN*) mRNA has a conventional AUG translation start site and a downstream alternative translation start site. Translation from the conventional start site results in the generation of the full-length OPN peptide, which includes a signal sequence that targets the nascent peptide to secretory vesicles, resulting in its secretion from the cell (sOPN). Translation from the alternative translation start site results in a short OPN peptide product that lacks a signal sequence and remains inside the cell (iOPN). ER, endoplasmic reticulum.

Figure 2. T-helper-cell polarization by osteopontin isoforms expressed by different cell types

The expression of the intracellular isoform of osteopontin (iOPN) is induced in plasmacytoid dendritic cells (pDCs) following ligation of Toll-like receptor 7 (TLR7) or TLR9, for example, during viral infection, and results in enhanced production of interferon- α (IFN α)⁹. This production of IFN α by pDCs contributes to T helper 1 (T_H1)-cell responses during viral infection. Activated T cells produce high levels of the secreted isoform of osteopontin (sOPN), which is under the control of the transcription factor T-bet². This triggers T_H1-cell responses through the induction of secretion of pro-inflammatory cytokines, including interleukin-12 (IL-12), by antigen-presenting cells¹. iOPN expressed by conventional DCs induces T_H17-cell responses by blocking the expression of IL-27, which suppresses the development of T_H17 cells. Type I IFN receptor (IFNAR) engagement suppresses the expression of iOPN, thereby removing the 'brake' on IL-27 expression and, consequently, on attenuated T_H17-cell responses¹¹.

Figure 3. Osteopontin-mediated regulation of pathogenic CD4⁺ T cells in experimental autoimmune encephalomyelitis

a | Dendritic cells (DCs) activate naive CD4⁺ T cells following the presentation of myelin antigen in peripheral lymphoid tissues. Inhibition of the expression of interleukin-27 (IL-27) by the intracellular isoform of osteopontin (iOPN) releases the ‘brake’ on T helper 17 (TH17)-cell development, resulting in a robust TH17-cell response¹¹. **b** | Activated T cells that migrate to the central nervous system (CNS) are re-stimulated by microglial cells, DCs and macrophages. Expression of iOPN by these antigen-presenting cells further enhances the TH17-cell response. At this stage, activated T cells produce high levels of the secreted OPN isoform (sOPN)², which promotes the survival of pathogenic TH1 and TH17 cells through the inhibition of apoptosis, resulting in sustained disease⁵⁷. **c** | Induction of type I interferon receptor (IFNAR)-mediated signalling in APCs following the administration of interferon-β (IFNβ) could result in the suppression of iOPN expression and upregulation of IL-27, which attenuates TH17-cell responses and, therefore, could inhibit the development of inflammatory disease in the CNS.