

- Berry, M.J., Banu, L., and Larsen, P.R. 1991. Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. *Nature*. **349**:438–440.
- Davey, J.C., Becker, K.B., Schneider, M.J., St. Germain, D.L., and Galton, V.A. 1995. Cloning of a cDNA for the type II iodothyronine deiodinase. *J. Biol. Chem.* **270**:26786–26789.
- Bianco, A.C., Salvatore, D., Gereben, B., Berry, M.J., and Larsen, P.R. 2002. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr. Rev.* **23**:38–89.
- Baqui, M.M.A., Gereben, B., Harney, J.W., Larsen, P.R., and Bianco, A.C. 2000. Distinct subcellular localization of transiently expressed types 1 and 2 iodothyronine deiodinases as determined by immunofluorescence confocal microscopy. *Endocrinology*. **141**:4309–4312.
- Bianco, A.C., and Silva, J.E. 1987. Intracellular conversion of thyroxine to triiodothyronine is required for the optimal thermogenic function of brown adipose tissue. *J. Clin. Invest.* **79**:295–300.
- Gereben, B., Goncalves, C., Harney, J.W., Larsen, P.R., and Bianco, A.C. 2000. Selective proteolysis of human type 2 deiodinase: a novel ubiquitin-proteasomal mediated mechanism for regulation of hormone activation. *Mol. Endocrinol.* **14**:1697–1708.
- Ravid, T., Doolman, R., Avner, R., Harats, D., and Roitman, R. 2000. The ubiquitin-proteasome pathway mediates the regulated degradation of mammalian 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J. Biol. Chem.* **275**:35840–35847.
- Curcio-Morelli, C., et al. 2003. Deubiquitination of type 2 iodothyronine deiodinase by von Hippel-Lindau protein-interacting deubiquitinating enzymes regulates thyroid hormone activation. *J. Clin. Invest.* **112**:189–196. doi:10.1172/JCI200318348.
- Berry, M.J., Maia, A.L., Kieffer, J.D., Harney, J.W., and Larsen, P.R. 1992. Substitution of cysteine for selenocysteine in type I iodothyronine deiodinase reduces the catalytic efficiency of the protein but enhances its translation. *Endocrinology*. **131**:1848–1852.
- Latif, F., et al. 1993. Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science*. **260**:1317–1320.
- Ohh, M., and Kaelin, W.G., Jr. 1999. The von Hippel-Lindau tumour suppressor protein: new perspectives. *Mol. Med. Today*. **5**:257–263.
- Li, Z., et al. 2002. Ubiquitination of a novel deubiquitinating enzyme requires direct binding to von Hippel-Lindau tumor suppressor protein. *J. Biol. Chem.* **277**:4656–4662.
- Li, Z., et al. 2002. Identification of a deubiquitinating enzyme subfamily as substrates of the von Hippel-Lindau tumor suppressor. *Biochem. Biophys. Res. Commun.* **294**:700–709.
- De Groot, L.J. 1999. Dangerous dogmas in medicine: the nonthyroidal illness syndrome. *J. Clin. Endocrinol. Metab.* **84**:151–164.
- Crantz, F.R., Silva, J.E., and Larsen, P.R. 1982. An analysis of the sources and quantity of 3,5,3'-triiodothyronine specifically bound to nuclear receptors in rat cerebral cortex and cerebellum. *Endocrinology*. **110**:367–375.
- Campos-Barros, A., et al. 1996. Phenolic and tyrosyl ring iodothyronine deiodination and thyroid hormone concentrations in the human central nervous system. *J. Clin. Endocrinol. Metab.* **81**:2179–2185.

## Osteopontin: a bridge between bone and the immune system

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The molecular mechanisms underlying the putative role of osteopontin in the chronic inflammatory disease rheumatoid arthritis are unclear. A study in a murine model of arthritis now demonstrates that a specific antibody directed against the exposed osteopontin epitope SLAYGLR (see the related article beginning on page 181) is capable of preventing inflammatory cell infiltration in arthritic joints.

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In recent years a number of studies have linked factors involved in inflammation to those critical for bone physiology and remodeling. One well described story is that of the receptor-activator of NF- $\kappa$ B ligand (RANKL)/osteoprotegerin (OPG) system, which plays a role in the

interaction between dendritic cells and T cells in the immune system and is the critical regulatory system for bone remodeling under physiologic conditions (1, 2). The RANKL/OPG system is also likely to play an important role in several forms of pathologic bone loss, including that seen in osteoporosis, certain forms of cancer, and inflammatory arthritis (3). There is mounting evidence for the role of another cytokine, osteopontin (OPN; “bone-bridging” protein), also known as early T cell activation gene-1 (Eta-1), in providing a link between the immune system and bone. In this issue of the *JCI*, Yamamoto et al. (4) provide important new evidence indicating a role for OPN in the pathogenesis of inflammatory arthritis and associated joint destruction.

### The role of OPN in bone and in the immune system

OPN is a phosphorylated glycoprotein secreted by activated macrophages, leukocytes, and activated T lymphocytes, and present in extracellular fluids, at sites of inflammation, and in the ECM of mineralized tissues (5, 6). This cytokine mediates important cell-matrix and cell-cell interactions. OPN is abundant in bone, where it facilitates the attachment of osteoclasts to the bone matrix via an interaction with cell surface  $\alpha$ v $\beta$ 3 integrin and CD44, the hyaluronic acid receptor (7). OPN<sup>-/-</sup> mice have a subtle bone phenotype, with delayed and impaired bone resorption (7). In the immune system, OPN plays a role in chemotaxis, leading to the migration of macrophages and dendritic cells to sites of inflammation. Activation of T lymphocytes results in an increase in OPN transcription, hence its alternative designation as Eta-1. Weber et al. have demonstrated that OPN is a T lymphocyte suppressor factor and that it enhances B lymphocyte Ig production and proliferation (8). In addition, OPN is an important cytokine mediating Th1 immunity (9).

OPN interacts with a variety of cell surface receptors, including the  $\alpha$ v $\beta$ 3,  $\alpha$ v $\beta$ 5,  $\alpha$ v $\beta$ 1,  $\alpha$ 4 $\beta$ 1,  $\alpha$ 8 $\beta$ 1, and  $\alpha$ 9 $\beta$ 1 integrins, as well as CD44. Binding of OPN to these cell surface receptors stimulates cell adhesion, migration, and specific signaling functions. The major integrin-binding site in OPN is the arginine-glycine-aspartate (RGD) integrin-binding motif, which is required for the

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**Nonstandard abbreviations used:** receptor-activator of NF- $\kappa$ B ligand (RANKL); osteoprotegerin (OPG); osteopontin (OPN); early T cell activation gene-1 (Eta-1); rheumatoid arthritis (RA); collagen-induced arthritis (CIA); collagen antibody-induced arthritis (CAIA).

adherence of many cell types to OPN. However, other sequences within OPN have also been shown to mediate cell adherence. For example, cleavage of human OPN by thrombin exposes the SVVYGLR sequence (SLAYGLR in the mouse), promoting the adherence of cells expressing  $\alpha 9$  and  $\alpha 4$  integrins.

### Potential role for OPN in the pathogenesis of inflammatory arthritis

Several prior studies have suggested an important role for OPN in the pathogenesis of inflammatory arthritis. OPN mRNA and protein have been demonstrated to be expressed in synovial tissues from patients with rheumatoid arthritis (RA), predominantly by fibroblastic cells, and at sites of pannus invasion into cartilage (10). In murine collagen-induced arthritis (CIA), OPN was detected in synovial tissues and at sites of osteoclast-mediated bone resorption, where its expression colocalized to sites of  $\alpha v \beta 3$  integrin expression (11). OPN has thus been implicated in the process of joint destruction in arthritis.

In their report in this issue of *JCI*, Yamamoto et al. (4) studied CIA and an animal model of RA known as collagen antibody-induced arthritis (CAIA). Arthritis was induced by the transfer of anti-type II collagen antibodies in C57BL/6 mice boosted with an intraperitoneal injection of LPS. These authors report that splenic monocytes from arthritic mice expressing  $\alpha 4$  and  $\alpha 9$  integrins demonstrated enhanced migration toward thrombin-cleaved OPN compared with splenic monocytes from nonarthritic mice. Of note is the fact that the ratio of the thrombin-cleaved form of OPN to noncleaved OPN was previously shown to be significantly increased in the plasma and synovial fluid of patients with RA compared with plasma from healthy controls and from patients with osteoarthritis (11). Furthermore, treatment of mice with an antibody (M5 Ab) directed against the sequence SLAYGLR, exposed by thrombin cleavage of murine OPN, inhibited synovitis and inflammatory cell infiltration into the joints of treated mice compared with those of arthritic control mice (4). M5 Ab treatment also resulted in protection from cartilage destruction in this murine model of RA.

In vitro studies were performed to demonstrate that the M5 Ab blocked the formation of osteoclast-like cells induced from bone marrow-derived precursors by treatment with RANKL and M-CSF. In addition, this antibody blocked the induction of calcium release from bone by parathyroid hormone (PTH) and IL-1 $\alpha$  in vitro (4). It has been previously demonstrated that PTH-induced bone resorption is dependent on OPN (12). These results suggest that one role of the M5 Ab in joint protection could be the blockade of osteoclast differentiation and function in vivo. A limitation of this study is that bone destruction was not directly assessed in the in vivo animal studies presented, and tartrate-resistant acid phosphatase-positive osteoclast-like cells were not quantitated in mice with and without M5 Ab treatment. In addition, since M5 Ab treatment inhibits joint inflammation in this animal model, protection from joint destruction may be a secondary phenomenon related to the general decrease in inflammation. Therefore a direct role of OPN in bone erosion in this model could not be determined. Given the important role of OPN in bone remodeling, such studies would be of great interest. Nonetheless, the effects on the clinical and histologic parameters studied provide convincing additional evidence for a role of OPN in arthritic inflammation, and specifically for its role in the recruitment of inflammatory cells to arthritic joints.

### Arthritis in the setting of OPN deficiency

Based on this information, one might expect that arthritis would be significantly attenuated in mice deficient in OPN. In fact this has been demonstrated in a CAIA model of RA (13) similar to that used in the study presented in this issue of *JCI* (4). OPN-deficient mice were found to have marked attenuation of joint swelling and articular cartilage destruction compared with arthritic wild-type mice and had no increase in urinary levels of deoxypyridinoline, a marker of bone destruction (13). These data support a role for OPN in both the inflammatory and the joint-destructive processes in arthritis. Interestingly, however, these results were called into question in a recent report in *Science*

(14) in which OPN was deleted by homologous recombination of strain 129-derived cells, and backcrossed into a CIA- and CAIA-susceptible strain for 12 generations. These authors then induced CIA and CAIA in the OPN-deficient mice and in littermates and demonstrated no effect of OPN deficiency in either form of murine arthritis. They concluded that prior observations in OPN-deficient mice showing protection from arthritis may have resulted from the deletion of polymorphic genes linked to OPN from strain 129, rather than from the deletion of OPN itself. These authors provided a list of several other genes within the deleted locus that could be important for arthritis pathogenesis.

The study of Yamamoto et al. (4) certainly adds to our understanding of the mechanisms by which OPN contributes to the pathogenesis of inflammatory arthritis. Given the clear and important role of OPN in inflammatory processes and bone remodeling, it will be of considerable interest to resolve some of the remaining controversies regarding the role of OPN in this disease.

1. Kong, Y.Y., et al. 1999. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature*. **397**:315–323.
2. Simonet, W.S., et al. 1997. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell*. **89**:309–319.
3. Pettit, A.R., and Gravalles, E.M. 2003. Osteoprotegerin. In *Targeted therapies in rheumatology*. P.E. Lipsky and J.S. Smolen, editors. Martin Dunitz, London, United Kingdom/New York, New York, USA. 359–377.
4. Yamamoto, N., et al. 2003. Essential role of the cryptic epitope SLAYGLR within osteopontin in a murine model of rheumatoid arthritis. *J. Clin. Invest.* **112**:181–188. doi:10.1172/JCI200317778.
5. Denhardt, D.T., and Noda, M. 1998. Osteopontin expression and function: role in bone remodeling. *J. Cell. Biochem. Suppl.* **30/31**:92–102.
6. Murry, C.E., Giachelli, C.M., Schwartz, S.M., and Vracko, R. 1994. Macrophages express osteopontin during repair of myocardial necrosis. *Am. J. Pathol.* **145**:1450–1462.
7. Chellaiah, M.A., et al. 2003. Osteopontin deficiency produces osteoclast dysfunction due to reduced CD44 surface expression. *Mol. Biol. Cell.* **14**:173–189.
8. Weber, G.F., Ashkar, S., Glimcher, M.J., and Cantor, H. 1996. Receptor-ligand interaction between CD44 and osteopontin (Eta-1). *Science*. **271**:509–512.
9. Jansson, M., Panoutsakopoulou, V., Baker, J., Klein, L., and Cantor, H. 2002. Attenuated experimental autoimmune encephalomyelitis in Eta-1/osteopontin-deficient mice. *J. Immunol.* **168**:2096–2099.
10. Petrow, P.K., et al. 2000. Expression of osteopontin messenger RNA and protein in rheumatoid arthritis: effects of osteopontin on the release of collagenase 1 from articular chondrocytes and synovial fibroblasts. *Arthritis Rheum.* **43**:1597–1605.

11. Ohshima, S., et al. 2002. Expression of osteopontin at sites of bone erosion in a murine experimental arthritis model of collagen-induced arthritis: possible involvement of osteopontin in bone destruction in arthritis. *Arthritis Rheum.* **46**:1094–1101.

12. Ihara, H., et al. 2001. Parathyroid hormone-induced bone resorption does not occur in the absence of osteopontin. *J. Biol. Chem.* **276**:13065–13071.

13. Yumoto, K., et al. 2002. Osteopontin deficiency protects joints against destruction in anti-type II collagen antibody-induced arthritis in mice. *Proc.*

*Natl. Acad. Sci. U. S. A.* **99**:4556–4561.

14. Blom, T., Franzen, A., Heinegard, D., and Holmdahl, R. 2003. Technical comments: comment on “The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease.” *Science.* **299**:1845a.

## Salicylic acid: an old dog, new tricks, and staphylococcal disease

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Aspirin has been shown to cause a reduction in the virulence of *Staphylococcus aureus*-associated endocarditis. A new study (see the related article beginning on page 222) reveals that salicylic acid, the major metabolite of aspirin, acts at the level of transcription to downregulate the production of fibrinogen, fibronectin, and  $\alpha$ -hemolysin — virulence factors necessary for bacterial replication in host tissues and, now, potential therapeutic targets.

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One hundred and twenty years after its initial description as the pathogen that causes sepsis and abscesses (1), *Staphylococcus aureus* remains a dangerous organism. Staphylococcal endocarditis is on the rise (2) and still causes significant mortality (3). The methicillin-resistant *S. aureus* (MRSA) epidemic has entered a new era due to the spread of MRSA into the community (4) and acquisition of new resistance cassettes with the potential for genetic transfer (5). The advent of fully vancomycin-resistant, methicillin-resistant clinical isolates (6) has further weakened the available armamentarium against this pathogen.

### Importance of staphylococcal attachment and invasion in endovascular disease

*S. aureus* is a nonmotile microorganism with a particular propensity to colonize biologic or artificial substrates using a

battery of pathogenicity factors (7), allowing for specific bacterial attachment. This can be followed by cellular invasion and subsequent tissue degradation. Several lines of evidence clearly indicate that the interaction with host proteins and platelets is instrumental in the development of disease. A plethora of bacterial factors — either wall bound (8) or secreted (9, 10) — mediate binding of and attachment to ECM molecules such as fibronectin, fibrinogen, collagen, and vWF. Work with deletion mutants and complemented heterologous hosts has demonstrated the particular role of adhesins that recognize fibronectin (such as fibronectin-binding protein A) and fibrinogen (such as clumping factor A, ClfA), allowing for cellular invasion and production of experimental endocarditis (11, 12), and *gfp* reporter assays from endocarditis models clearly indicate that activation of global regulators that coordinate adhesin and toxin expression, such as *agr* and *sar*, occurs in vivo (13, 14).

### Distinctive effects of acetylsalicylic acid and salicylic acid on platelets and bacteria

A particular role of platelets in the pathogenesis of staphylococcal endocarditis has been suggested since the early observation by Durack of bacterial interaction with fibrin-platelet matrices

at sites of nonbacterial thrombotic endocarditis (15) and the series of reports by Clawson et al. on the interaction of *S. aureus* with purified platelets (16). In the early 1990s, experiments with surface-activated platelets suggested to our group the importance of fibrinogen and *S. aureus* clumping factor in the bacteria-platelet interaction (17). These observations were subsequently confirmed and extended by use of a low-platelet-binding mutant expressing a mutated ClfA protein (18) that displays diminished virulence in an endocarditis model (19), and by identification of the secreted fibrinogen-binding proteins Coa and Efb in phage-display panning assays (20) (Figure 1).

While these observations pointed toward complex but, according to their adhesive function, rather pro-pathogenic events at the bacteria-endocardium interface, the role of platelets had to be reevaluated after the discovery that they function as specialized inflammatory cells (21) in response to secretion of antimicrobial peptides. In fact, paradoxically, hyperexpression of  $\alpha$ -toxin by *S. aureus* results in diminished virulence in experimental endocarditis, possibly because of the release of platelet microbicidal proteins (22).

The attributed role of platelets in the disease process that results in endovascular infection has prompted a number of researchers to interfere with platelet function for prevention or treatment of endocarditis. Acetylsalicylic acid (ASA, aspirin) has been used in vitro and in a number of experimental models to reduce vegetation sizes and to mitigate the course of disease (23–25). Similar effects have also been observed by Kupferwasser et al. (26). However, when they studied its metabolite, salicylic acid (SAL), in parallel to ASA, they made the interesting observation that pretreatment of bacteria with SAL reduced attachment to the valvular epithelium to an even greater extent than administration of ASA. This observation was accompanied by the in vitro finding that SAL-pretreated *S. aureus* cells bound to

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**Nonstandard abbreviations used:** clumping factor A (ClfA); acetylsalicylic acid (ASA); salicylic acid (SAL).