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The Biology of Aseptic Osteolysis

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Total hip arthroplasty is one of the most commonly performed and successful elective orthopaedic procedures. However, numerous failure mechanisms limit the long-term success including aseptic osteolysis, aseptic loosening, infection, and implant instability. Aseptic osteolysis and subsequent implant failure occur because of a chronic inflammatory response to implant-derived wear particles. To reduce particulate debris and their consequences, implants have had numerous design modifications including high-molecular–weight polyethylene sockets and noncemented implants that rely on bone ingrowth for fixation. Surgical techniques have improved cementation with the use of medullary plugs, cement guns, lavage of the canal, pressurization, centralization of the stem, and reduction in cement porosity. Despite these advances, aseptic osteolysis continues to limit implant longevity. Numerous proinflammatory cytokines, such as interleukin-1, interleukin-6, tumor necrosis factor-alpha, and prostaglandin E_2, have proosteoclastogenic effects in response to implant-derived wear particles. However, none of these cytokines represents a final common pathway for the process of particle-induced osteoclast differentiation and maturation. Recent work has identified the fundamental role of the RANKL-RANK-NF-κB pathway not only in osteoclastogenesis but also in the development and function the immune system. Thus, the immune system and skeletal homeostasis may be linked in the process of osteoclastogenesis and osteolysis.

Total hip arthroplasty (THA) is one of the most successful and effective procedures developed for treatment of pain associated with end-stage arthritis. Long-term followup studies report a survivorship greater than 80% at 20 years after surgery.[7,12,66,113] As a result of this success, there has been an increasing number of prostheses implanted into younger, more active individuals.[50] This increased functional demand is coupled with increased implant wear rates with standard bearing materials.[14,26,64,111,121] Subsequently, there are numerous mechanisms of failure that limit the long-term success of hip arthroplasty surgery. Aseptic failure is the most common reason for implant failure accounting for approximately 75% of cases. The next most common reasons for implants requiring revision are infection (7%), recurrent dislocation (6%), periprosthetic fracture (5%), and technical errors at the time of surgery (3%).[13,24,75,77,100,109] Aseptic failure occurs secondary to a chronic, granulomatous, inflammatory response that is stimulated by and maintained by implant-derived wear particles.[100,109]

The process of particle-induced osteolysis is complex and involves the interaction of numerous cytokines, chemokines, growth factors, and cell types. Our understanding of this process has been greatly advanced by the discovery of the RANK-RANKL pathway and the fundamental role that it plays in physiologic and pathologic osteoclast function. Although hydrostatic fluid pressure may accentuate experimentally induced cells to bone resorption,[56,82,83] particle-induced osteolysis is considered the principal cause.

In this review, we discuss the importance of the RANK-RANKL pathway with relevance to particle-induced osteoclastogenesis and bone resorption. In addition, we discuss recent research into the pharmacologic modulation of this process and the potential role of interleukin (IL)-18.

Search Strategy

To identify relevant literature, we used a sensitive search of the most common databases of published medical literature, including Ovid MEDLINE®, Cochrane Database of Systematic Reviews, EMBASE, BMJ Clinical Evidence, and CINAHL. The search strings used was osteolysis(TI) and one or more of the following key words:

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hip, replacement, loosening, implant, arthroplasty, aseptic, and particle.

We made no restrictions on language or date. A final check that no relevant articles were missed was conducted by screening the references from the articles that had been selected.

Implant Design

All articulating mechanical devices undergo wear at contact interfaces. Ultra-high molecular-weight polyethylene (UHMWPE) has been one of the most frequently used bearing surfaces in the majority of hip arthroplasty designs. Alternative bearings have been developed in an attempt to avoid particle-induced osteolysis.[50] More highly crosslinked polyethylenes have been introduced to reduce wear, and data support this in vitro and in vivo.[9,23,54,104] However, some highly crosslinked polyethylenes may give rise to an increased proportion, per unit volume of wear, of wear particles in the critical size range for macrophage activation.[29,59] Ceramic-on-ceramic bearings have had extremely low wear rates even under adverse microseparation and edge loading conditions.[46,94] However, alumina particles produced under such conditions are capable of inducing osteolytic cytokine production in vitro, but relatively high concentrations are required.[48] Metal-on-metal articulations produce much smaller wear particles in the nanometer size range (20–90 nm)[25,93] Particles in this size distribution do not directly stimulate macrophages but represent a very high surface area of exposure and do result in elevated Co and Cr ion levels.[43] The long-term potential side effects of elevated serum metal ions levels are as yet unknown but remain a cause for concern. Recent work by Hart et al[47] showed that metal-on-metal articulations are associated with a decrease in CD8+ve T cell counts. However, no adverse effects were reported as a consequence of this reduced cell count. Despite the small particle distribution of wear debris from metal-on-metal articulations, periimplant osteolysis does occur.[61] These ions may up-regulate T lymphocyte function and expression of RANKL, thus predisposing macrophages to respond to relatively small particle loads.[44] However, the exact role of T lymphocytes in particle-induced osteolysis has yet to be determined.

Wear Debris Morphology

Particles retrieved from failed THAs are known to vary in size and morphologic features depending on implant design. Ultra high molecular weight particles isolated from failed hip implants vary in shape from spheroids to fibrils and in size from 0.1 μm to several millimeters.[13,76,77,80,120] Macrophages appear most sensitive to submicron-sized particles, with giant cells forming around larger (larger than 10 μm) particles (Fig 1).[76,77] The volume of particulate polyethylene debris was underestimated in early studies of periprosthetic tissues. As light-microscopy resolution is limited by the wavelength of visible light (0.4–0.7 μm), submicrometer particles cannot be seen well with these techniques. Studies of wear debris from periprosthetic tissues by scanning electron microscopy have shown that 70% to 90% of the recovered particles are submicrometer, with a mean size of approximately 0.5 μm.[76,77]

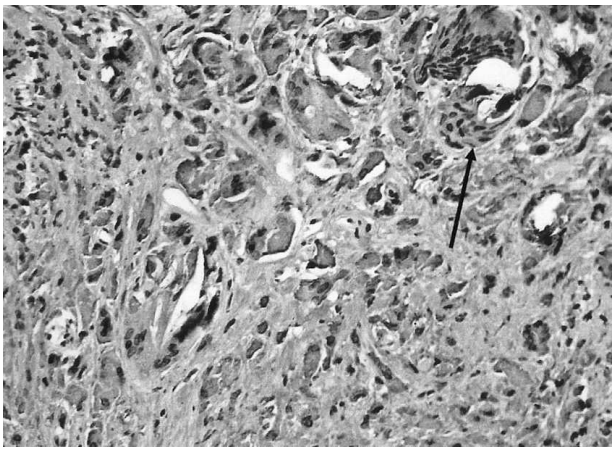

Fig 1. This photomicrograph shows a section of pseudomembrane obtained from a patient having revision surgery for a failed THA secondary to aseptic osteolysis. The 5-μm formaldehyde-fixed paraffin section was stained with an anti-CD68 antibody using a standard streptavidin-horseradish peroxidase protocol. The product was viewed with 3,3'-diaminobenzidine tetrachloride and counterstained with hematoxylin (original magnification, ×200). The arrow indicates CD68-positive giant cells surrounding a UHMWPE particle.

The bioreactivity of particles depends on their size, composition, and concentration.[19] Particles that are phagocytosable are the most stimulatory and increasing doses elicit a more marked response (up to a saturation or toxic level). All particle types can elicit a cell response; however, as yet it has not been determined which particle types have the greatest clinical effect.

Bone Homeostasis

To fully understand how particle-induced osteolysis develops, we must first consider the delicately balanced process of skeletal homeostasis. Skeletal bone undergoes continuous remodeling by a process that involves the resorption of bone by osteoclasts coupled with the synthesis and deposition of new bone matrix by osteoblasts.[31,105] Osteoblasts and osteoclasts arise from distinct cell lines. Osteoblasts arise from mesenchymal stem cells, whereas osteoclasts differentiate from hematopoietic monocyte/macrophage precursors.[79,101,126] The osteoclast

is a polykaryocyte formed by the fusion of mononuclear cells derived from hematopoietic bone marrow precursors. The differentiation of myeloid progenitor cells into committed osteoclast lineage is characterized by the appearance of the mRNA and protein for vitronectin receptor ($\alpha v\beta 3$), cathepsin K, tartrate-resistant acid phosphatase (TRAP), and calcitonin receptor.[89,105] The appearance of this receptor is followed closely by the acquisition of bone-resorbing capacity.

Many cytokines affect osteoclastogenesis at distinct stages of development, including colony-stimulating factor-1 (CSF-1; also known as M-CSF), IL-1, transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α), IL-6, vitamin D_3, IL-11, calcitonin, prostaglandin E_2 (PGE_2), and parathyroid hormone.[42,89,105] However, genetic ablation experiments suggest these factors are not essential for osteoclast development in vivo.[21,69] Imbalances between osteoclast and osteoblast activity arise as a consequence of disturbance of controlling cytokines, which can lead to a localized or systemic reduction in bone mass.[89,103] Increased osteoclast activity is seen in many osteopenic disorders, including postmenopausal osteoporosis, Paget's disease, lytic bone metastases, and rheumatoid arthritis.[65,106,118]

Biologic Response to Implant-derived Wear Particles

Aseptic failure of a hip prosthesis occurs as a consequence of a chronic, granulomatous, inflammatory response, which results in the formation of a pseudomembrane at the bone-cement-prosthesis interface.[39,56,109] This tissue is unique to the setting of aseptic implant failure and is characterized by the presence of macrophages, fibroblasts, foreign body giant cells, and lymphocytes in a connective tissue matrix.[34,37,39,76] A vast array of cytokines, chemokines, and growth factors has been isolated from the pseudomembrane that surrounds failed implants. These include TNF-α, IL-1β, IL-6, IL-8, IL-11, TGF-β, PGE_2, CSF-1, granulocyte-macrophage colony-stimulating factor (GM-CSF), and matrix metalloproteinases (MMPs).[1,2,15,40,63,68,107,125,134,135] These factors may stimulate osteoclast differentiation and maturation (TNF-α, IL-1) or may be responsible for bone matrix degradation (MMPs). Exactly how each of these various mediators is involved in particle-induced osteolysis has yet to be fully ascertained; however, there appears to be a self-perpetuating inflammatory response driven largely by the macrophage in response to biologically active particles.

Numerous researchers have conducted studies using in vivo and in vitro models of particle-induced osteoclastogenesis to determine the effects of particulate wear debris on the functions of macrophages, fibroblasts, and osteoclasts.[19–21,58,59]

To discuss each of the cytokines listed in detail is out of the scope of this article; however, we will discuss two of the most important, namely, TNF-α and IL-1.

TNF-α

TNF-α is a potent osteoclastogenic cytokine that has been identified as having a fundamental role in the pathogenesis of implant particle-induced osteolysis.[8,21,95,99,102] This cytokine is present in periprosthetic membrane tissue obtained from loose implants,[15,63,134] and macrophage expression of TNF-α in vitro is increased by exposure to implant-associated particles.[8,39,49,51,76,85,114] Tumor necrosis factor-α is a regulatory cytokine for other proinflammatory cytokines including IL-1, IL-6, IL-8, and GM-CSF.[15,132] Tumor necrosis factor-α is known to act synergistically with RANKL in stimulating osteoclastogenesis, and the signal transduction cascade used by the TNF receptor results in NF-κB activation.[85] Genetic and pharmacologic blockade of TNF-α signaling dampens polymethylmethacrylate (PMMA) particle-induced osteolysis; however, the process still takes place.[21,114] These findings point toward the TNF-α signaling pathway as playing a major role in implant particle-stimulated bone loss via activation of NF-κB.

IL-1

Interleukin-1 is a proinflammatory cytokine, the activities of which include induction of fever, expression of vascular adhesion molecules, and roles in arthritis and septic shock.[132] The inflammatory activities of IL-1 are partially derived by inducing expression of cytokines such as TNF-α.[132] The signal transduction cascade used by IL-1 receptor is similar to that of TNF-α, resulting in NF-κB activation.

Interleukin-1 is an osteoclastogenic cytokine and promotes RANKL expression by marrow stromal cells and osteoblasts.[139] Interleukin-1 promotes multinucleation of osteoclast precursors and enhances the capacity of the mature polykaryon to resorb bone. Tumor necrosis factor-α induction of RANKL expression by marrow stromal cells is substantially mediated by IL-1 via enhanced expression of IL-1RI. Like TNF- α, IL-1 has the capacity to directly target mononuclear osteoclast precursors and promote their differentiation but requires RANKL to do so.[132,139] Thus, IL-1 is a key downstream effector molecule in TNF-α-induced osteoclastogenesis, participating in stimulated RANKL expression by stromal cells and direct targeting of osteoclast precursors. Interleukin-1 has been identified from cultured macrophages in response to PMMA and titanium particles.[36,37] It also has been identified in the periprosthetic pseudomembrane isolated from individuals undergoing revision surgery for aseptic loosening.[68]

Again, like TNF-α, IL-1 is a proosteoclastogenic cytokine but is not essential for osteoclastogenesis.[139]

Although numerous cytokines have been identified as having proosteoclastogenic effects, none of these cytokines represents a final common pathway for the process of osteoclast differentiation and maturation. The inflammatory pathways that drive particle-induced osteolysis are complex and have interrelated tracks, providing a redundancy in the inflammatory cascade. As such, blockade of one cytokine such as TNF-α will not abolish the proinflammatory response. However, studies have identified the fundamental role of the RANKL-RANK interaction in particle-induced osteoclastogenesis.[19–21]

RANKL-RANK-OPG-NF-κB

Our basic understanding of osteoclast biology and the molecular mechanisms of osteoclast differentiation has been advanced by the identification of the importance of the RANK-RANKL signal transduction pathway (Fig 2). We will now discuss the important molecules/receptors involved in the regulation of this pathway with particular reference to NF-κB, RANKL, RANK, and OPG.

NF-κB

Nuclear factor kappa-B (NF-κB) is a nuclear transcription factor that regulates expression of a large number of genes that are critical for regulation of cellular apoptosis, inflammation, and autoimmune disease. The activation of NF-κB is thought to be part of a stress response as it is activated by various stimuli that include growth factors, cytokines, pharmacologic agents, and stress.[69] In its inactive form, NF-κB is sequestered in the cytoplasm, bound by inhibitor proteins. The various stimuli that activate NF-κB cause degradation of these inhibitory proteins, which results in translocation of the activated molecule to the cell nucleus. Nuclear factor-κβ binds with sequence genes and activates their transcription, leading to the production of cytokines and growth factors that control many processes such as osteoclastogenesis.

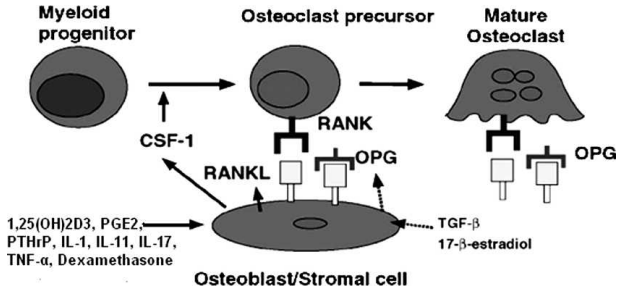

Fig 2. A diagram illustrates regulation of osteoclast formation in bone tissues. Calciotropic factors such as PGE_2, IL-1, and TNF-α induce RANKL expression on osteoblasts. RANKL binding to RANK expressed on hematopoietic progenitors activates a signal transduction cascade that leads to osteoclast differentiation in the presence of CSF-1. Additionally, RANKL stimulates bone-resorbing activity in mature osteoclasts via RANK. Osteoprotegerin produced by osteoblasts acts as a decoy receptor for RANKL and inhibits osteoclastogenesis and osteoclast activation by binding to RANKL. 1,25(OH)2D3 = 1,25-dihydroxy-vitamin D_3; PTHrP = human parathyroid hormone-related peptide receptor.

Nuclear factor-κB is central to osteoclast differentiation. In fact, deletion of the p50 and p52 NF-κB subunits completely arrests osteoclastogenesis, resulting in severe osteopetrosis.[60,69,137]

The NF-κB signaling pathway has been identified as a potential mediator of particle-induced osteoclastogenesis and osteolysis.[19–21,92,116] Nuclear factor-κB is activated on implant particle exposure in various macrophage cell systems.[20] Polymethylmethacrylate particles rapidly induce NF-κB DNA binding affinity in murine osteoclast precursor cells (CSF-1-dependent bone marrow macrophages).[20,21] Additionally, human macrophages exposed to titanium particles obtained from periprosthetic membranes display rapid (less than 30 minutes) activation of NF-κB.[92] Blockade of particle phagocytosis by cytochalasin B did not inhibit activation of NF-κB, suggesting particle contact with macrophage cell surface membrane proteins is sufficient to stimulate transcription factor activation.[19] Clohisey et al[20] used NF-κB signaling blockade to determine the action of this pathway in PMMA particle-induced osteoclastogenesis in vitro. Nuclear factor-κB inhibitors effectively block particle-induced NF-κB DNA binding activity, nuclear translocation of the active NF-κB p50 subunit, and osteoclastogenesis in vitro. Thus, the NF-κB signaling mechanism also may be fundamental to implant particle-induced osteoclastogenesis and periprosthetic osteolysis.

RANKL

Receptor activator of nuclear transcription factor-kappa B ligand (RANKL; also known as TRANCE/ODF) is a member of the TNF-α superfamily and is essential for osteoclastogenesis.[11,52,69,70,117,127,138] RANKL exists predominantly as a membrane-anchored protein requiring cell-to-cell contact to lead to NF-κB activation. However, it may be released from the cell surface after proteolytic cleavage.[74] RANKL is expressed extensively in mature osteoblasts, osteoblast precursor stromal cells, primitive mesenchymal cells, hypertrophied chondrocytes, and activated T lymphocytes. RANKL expression can be upregulated by numerous molecules (Table 1).[70,138] In in vitro culture systems, RANKL can activate mature osteoclasts and mediate osteoclastogenesis from myeloid precursors in the presence of CSF-1. It also acts to inhibit osteoclast apoptosis.[33,70,138]

TABLE 1. Molecules That Regulate RANKL and OPG Levels[65,68]

Molecule	RANKL	OPG
Hormones		
Vitamin D_3	Increased	Increased
PTH/PTHrP	Increased	Decreased
Estradiol	No change	Increased
Cytokines		
TNF-α	Increased	Increased
IL-1	Increased	Increased
IL-6	Increased	Unknown
IL-11	Increased	Unknown
IL-17	Increased	Unknown
IL-18	Decreased	Increased
Growth factors		
TGF-β1	Decreased	Increased
BMP-2	Unknown	Increased
Others		
Prostaglandin E_2	Increased	Decreased
Glucocorticoid	Increased	Decreased

RANKL = receptor activator of NF-κB ligand; OPG = osteoprotegerin; PTH/PTHrP = human parathyroid hormone/PTH-related peptide receptor; TNF-α = tumor necrosis factor-alpha; IL = interleukin; TGF-β1 = transforming growth factor-beta; BMP-2 = bone morphogenic protein-2

RANKL-deficient mice have severe osteopetrosis, stunted growth, a defect in tooth eruption, and RANKL-deficient osteoblasts that cannot support osteoclastogenesis. However, these mice contain hematopoietic precursors that can differentiate into phenotypically and functionally mature osteoclasts in vitro in the presence of recombinant RANKL and CSF-1. Importantly, osteoblast cell lines derived from RANKL-deficient mice do not support osteoclast formation, indicating the defect in osteoclastogenesis observed in these mice is attributable to an intrinsic defect in osteoblastic stroma. Thus, RANKL is a specific and essential differentiation factor for osteoclast precursors and an activation factor for mature osteoclasts.[69,129]

RANKL also has important roles in the development and regulation of the acquired immune system and is involved in regulating T cell/dendritic cell communications, dendritic cell survival, and lymph node organogenesis.[69,129] Production of RANKL by activated T cells can directly regulate osteoclastogenesis and bone remodeling, and it explains why autoimmune diseases, cancers, leukemias, asthma, chronic viral infections, and periodontal disease result in systemic and local bone loss.[69,88,129] It also may explain a possible pathway for metal wear particles to stimulate osteolysis.[61] In particular, overexpression of RANKL by activated T cells appears to be the pathogenic principle that causes bone and cartilage destruction in arthritis.[129] Collectively, the overall action of this ligand is to expand the pool of mature, activated osteoclasts and thus shift local or systemic bone homeostasis to increased bone resorption.

RANK

Receptor activator of NF-κB (RANK; also known as TRANCE-R) is a TNF-α superfamily transmembrane receptor molecule expressed on the cell surface of hematopoietic osteoclast precursors, mature osteoclasts, dendritic cells, chondrocytes, and mammary gland epithelial cells.[3,57,71] Binding of RANK with its ligand RANKL initiates a signaling cascade that leads to the differentiation of osteoclast precursors and activation of mature osteoclasts.[11,57,91] Mice with a genetic mutation of RANK have a complete block in osteoclast development that can be restored by reintroduction of RANK into bone marrow progenitor cells.[27,71,91]

OPG

The biologic activity of RANKL is regulated by a soluble protein named osteoprotegerin (OPG; also known as osteoclastogenesis inhibitory factor). Osteoprotegerin is a member of TNF-α receptor superfamily and is secreted as a soluble protein by bone marrow stromal cells and osteoblasts.[137] Osteoprotegerin is a competitive inhibitor of RANKL, binding to the RANK receptor on the cell surface of osteoclast precursor cells and mature osteoclasts. By blocking the RANKL-RANK interaction, it counteracts the proosteoclastogenic actions of RANKL and therefore forms an essential part of the biomolecular control mechanisms between osteoclast and osteoblast cell lines that regulate bone homeostasis.[70,122,137,138]

Inhibition of RANKL function via OPG prevents bone loss in animal models of tumor metastases and arthritis. High systemic levels of OPG in OPG transgenic mice cause osteopetrosis with abnormal tooth eruption and bone elongation, and these levels also inhibit the development and activity of endosteal osteoclasts.[122] In contrast, OPG-deficient mice display severe osteoporosis associated with a high incidence of fractures, indicating the level of bone mass correlates with the levels of OPG in mice.[10,87]

Osteoprotegerin expression also is induced by estrogen in cell lines and in vivo,[53,108] explaining postmenopausal osteoporosis in women: reduced ovarian function leads to reduced estrogen levels and hence reduced OPG levels, which release RANKL from the inhibition by the decoy receptor. Injection of OPG into ovariectomized rats blocks bone loss and osteoporosis normally associated with the loss of ovarian function.[27] Thus, OPG and/or modulation of RANKL-RANK function via small molecules are promising avenues to prevent postmenopausal osteoporosis.

Injection of OPG prevents osteoclast activation and osteopenia in essentially every model system of osteoclast-mediated bone loss. All factors that inhibit or enhance bone resorption via osteoclasts act by regulation of RANKL-RANK and/or OPG (Table 1). Therefore it ap-

pears that the complex system of osteoclast-regulated bone remodeling is controlled by these three molecules.

Role of the RANKL-RANK Signaling Pathway in Particle-induced Osteolysis

Numerous investigators have studied the components of the RANK-RANKL-NF-κB signaling pathway to determine their role in particle-induced osteoclastogenesis.[18–21,33,38,57,60] Clohisy et al,[19] using a murine whole bone marrow cell culture model, found RANKL in its soluble form was present in the culture medium and observed a 2.5-fold increase during stimulation with particles of PMMA. Subsequent experiments involving cultures of pure macrophage osteoclast precursors devoid of supporting stromal cells showed, in the absence of exogenous RANKL, these cultures showed no osteoclastogenic potential after either PMMA or TNF-α stimulation. In contrast, addition of RANKL to these cultures enabled basal TNF-α and particle-stimulated osteoclastogenesis. This experiment suggests stromal cell-derived RANKL is essential for basal and PMMA-induced osteoclastogenesis.

The fundamental role of RANK-RANKL binding is further underscored by in vivo and in vitro work using RANK:Fc antibodies that competitively block RANKL from binding to its receptor. RANK signaling blockade with RANK:Fc prevents experimental particle-induced osteolysis in an in vivo murine model.[18,19]

In addition, the decoy receptor OPG inhibits in vitro murine osteoclast formation induced by fluid from failed THAs.[67] Other investigators also established OPG as an effective in vivo inhibitor of particle-induced osteolysis in a dose-dependent and reversible manner.[19]

Particle exposure not only directly affects bone homeostasis by stimulating osteoclastogenesis but also has major effects on osteoblast function and gene expression. Osteoblast exposure to particulate matter results in decreased alkaline phosphatase and Type I collagen synthesis and enhanced expression of certain proinflammatory factors including IL-6 and PGE_2.[73,131] Similarly to TNF-α, stromal- and osteoblast-derived IL-6 and/or PGE_2 in conjunction with RANKL may further enhance the particle-induced osteoclastogenesis.[112]

Tissue Explant Studies

The results of in vivo and in vitro animal models do not always mirror those obtained when studying human subjects. Even using human-derived in vitro models cannot replicate the conditions that occur during the process of particle-induced osteolysis. However, immunohistochemical analysis of the periimplant pseudomembrane excised from patients with implant-induced osteolytic implant failure had higher levels of RANKL protein than that found in similar tissues from control subjects. In contrast, OPG protein levels were similar in both groups.[22] RANKL mRNA and protein were associated predominantly with cells containing wear particles. Dual labeling studies showed the cells expressing RANKL protein were macrophages. In situ hybridization studies confirmed mRNA encoding for these proteins also is expressed by cells in the periimplant tissues.[4] In addition, RANK mRNA was expressed in cells that contained wear particles.[78] These findings show abnormally high levels of RANKL are expressed in periimplant tissues of patients with prosthetic loosening and these abnormal levels of RANKL may contribute substantially to aseptic implant loosening.[4,22,78]

An Immunologic Basis for Particle-induced Osteolysis?

Numerous cells involved in the pathogenesis of particle-induced osteolysis form part of the immune system. Several investigators have studied the role of the specific immune system to determine whether particulate matter induces a specific immunologic response. Santavirta et al[110] cultured peripheral blood mononuclear cells with PMMA particles and found induction of an inflammatory nonspecific lymphocyte reaction and concluded PMMA is essentially immunologically inert. Using a human lymphocyte culture to assess the biocompatibility of UHMWPE, the same authors concluded, while the particles induced a foreign-body reaction, they were also immunologically inert.

Total hip arthroplasty with metal-on-metal bearings was introduced as an alternative to the use of metal-on-polyethylene bearings because of such theoretical advantages as reduced wear and a lower prevalence of osteolysis. Numerous issues cause concern with metal-on-metal bearings, including metal ion exposure and the potential for metal hypersensitivity. All metals that are in contact with biologic systems corrode, and the released ions can activate the immune system by forming metal-protein complexes.[45,62,81] Metal ions are allergens that are known to cause contact dermatitis, a delayed-type hypersensitivity reaction mediated by T lymphocytes specific for the relevant metal.[45,81] Allergic reactions also can occur in the joint capsule and pericapsular tissue.[81] The most common metal sensitizer in humans is nickel, followed by cobalt and chromium, and cross-sensitivity reactions between metals are most common with nickel and cobalt.[45,62] Several studies have reported aseptic failure of metal-on-metal hip prostheses in association with hypersensitivities to cobalt, nickel, and chromium.[6,28,30] The prevalence of metal sensitivity among patients with a well-functioning implant is approximately twice that in the general population, and among patients with a failed or poorly functioning implant, it is approximately six times that in the general population.[45] However, it has yet to be determined wheth-

er there is a causative relationship between metal hypersensitivity and implant failure.

IL-18

Interleukin-18 is a novel cytokine that may play a substantial role in the disturbance in bone homeostasis observed in numerous systemic disorders, including inflammatory arthritis and metastatic skeletal deposits.[72,136] However, as yet no in vivo or in vitro studies have examined the role of IL-18 in particle-induced osteoclastogenesis. Interleukin-18 is a member of the IL-1 cytokine family. Its precursor molecule, pro-IL-18, is cleaved by caspase-1 to yield an active 18-kDa glycoprotein.[35] Initially characterized by its capacity to promote Th1 responses in synergy with IL-12,[90] IL-18 also has been ascribed broader properties in the acquired immune response.[123,133] It induces Th1 cell proliferation, IL-12Rα expression, and interferon-gamma (IFN-γ), TNF-α, and GM-CSF production by Th1 clones.[96] However, at early stages of T cell differentiation, IL-18 can promote either Th1 or Th2 responses, suggesting a broader role in functional T cell differentiation than that originally recognized.[133] In keeping with an early role in immune responses, IL-18 mRNA is widely distributed, facilitating rapid generation of cytokine if required. Synthesis of IL-18 protein has been described thus far in macrophages, Kupffer cells, keratinocytes, fibroblasts, chondrocytes, and osteoblasts.[41,97,98,123,124,130]

A Role for IL-18 in Implant-induced Osteolysis?

Ongoing work from our group (unpublished) considers the possible role of IL-18 in particle-induced osteolysis. Pseudomembrane tissue samples excised from individuals with loose joint arthroplasties were obtained from five patients having revision surgery. This tissue was paraffin fixed and sectioned into 7 μm sections before undergoing immunohistochemical and polymerase chain reaction (PCR) analysis. Interleukin-18 protein and mRNA were identified in all tissue samples (Fig 3).

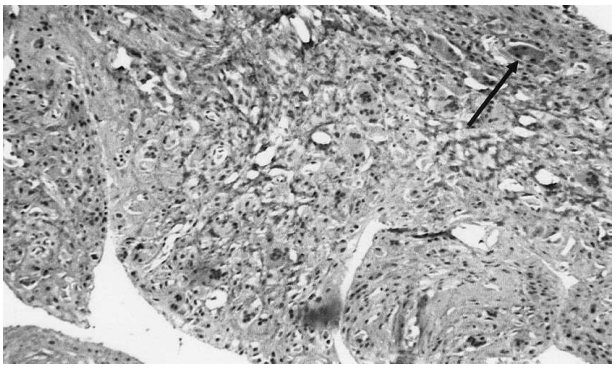

Fig 3. This photomicrograph shows a section of pseudomembrane obtained from a patient having revision surgery for a failed THA secondary to aseptic osteolysis. The 5-μm formaldehyde-fixed paraffin section was stained with an anti-IL-18 antibody using a streptavidin-horseradish peroxidase protocol. The product was viewed with 3,3'-diaminobenzidine tetrachloride and counterstained with hematoxylin (original magnification, ×200). The arrow indicates a multinucleate cell staining positive for IL-18.

An in vitro model of PMMA-induced osteoclastogenesis was created using whole bone marrow cultures obtained by isolating marrow from the tibia and femurs of 8- to 10-week-old BALB/c female mice. Cells were plated in Nunclon™ tissue culture plates (Nalge Nunc International, Rochester, NY) and incubated at 37°C in 5% CO_2 in an osteoclastogenic growth medium consisting of Dulbecco's modified Eagle's medium (Sigma-Aldrich, UK) supplemented with 10% fetal calf serum, 2 mmol/L glutamine, 10 nmol/L 1,25-dihydroxy-vitamin D_3, 100 IU/mL penicillin, 100 mg/mL streptomycin, and 1.25 μg/mL amphotericin B. Cells were cultured for 8 days with medium being exchanged every other day. On Day 8, cells were subjected to numerous experimental conditions. Commercially available spherical PMMA particles 1 to 10 μm in diameter (mean diameter, 4.5 μm; 95% < 10 μm) were used in all experiments. This particle size distribution is within the biologically active range, as PMMA particles in the 1 to 300 μm distribution are known to stimulate macrophage activity in vitro. Supernatant, RNA, and nuclear extract were taken at baseline and at 48 hours. Osteoclastogenesis was determined by counting cells staining positive for TRAP with three or more nuclei. Osteoclast activity was assessed by calculating the percentage of surface resorption of dentine slices. Each dentine slice was stained with toluidine blue and photographed. Image analysis software was used to calculate percentage resorption by threshold analysis. Relevant expression of cytokines, including TNF-α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-17, GM-CSF, and interferon-gamma (INF-γ), was analyzed using the Luminex® laser bead antibody system (Luminex Corp, Austin, TX). Interleukin-18, soluble RANKL, OPG (and TGF-β) were analyzed by ELISA. Nuclear factor-κB expression was analyzed by performing ELISA of cell nuclear extract.

We have showed IL-18 is a potent inhibitor of PMMA-induced osteoclastogenesis in vitro as seen by reduced dentine disc resorption (Fig 4) and TRAP-positive osteoclast cell counts from murine whole bone marrow tissue cultures. Neutralization of endogenous IL-18 using a neutralizing antibody resulted in increased osteoclastogenesis in vitro. Increased levels of OPG protein and mRNA were noted in samples stimulated with IL-18. As yet we have not determined the origin of the OPG. No OPG was detected in samples with IL-18-neutralizing antibody. Therefore, at least in vitro, IL-18 may have an important role in regulating particle-induced osteoclastogenesis.

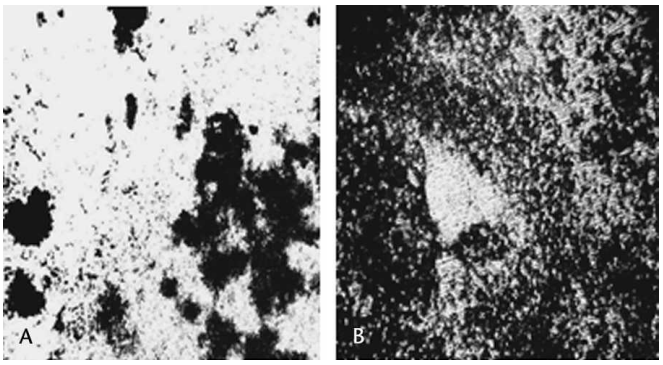

Fig 4A–B. Dentine disc samples show IL-18 is a potent inhibitor of PMMA-induced osteoclastogenesis. (A) The sample treated with IL-18 shows IL-18-induced inhibition of osteoclast pit formation compared with (B) the control sample. Both samples had PMMA particle stimulation.

Pharmacologic Modulation of Particle-induced Osteolysis

Our improved understanding of the cytokines and receptors that influence particle-induced osteolysis has led to the trial of novel agents with the aim of pharmacologically modulating this process.

Bisphosphonates

The bisphosphonates are a class of drug that are analogues of pyrophosphate. They are commonly used for medical treatment of osteoporosis, Paget's disease, and hypercalcemia. Numerous in vivo and in vitro animal model studies suggest these agents may be of potential benefit for treatment of particle-induced osteolysis.[55,86,119,128] These agents inhibit osteoclast function downstream and do not affect the particle-induced inflammatory response. There are no significantly powered randomized, controlled trials as yet that evaluate the bisphosphonates for treatment/prevention of particle-induced osteolysis, representing an area for future study.

Anti-TNF-α Therapy

We already have discussed the important role this cytokine plays in the inflammatory response to implant-derived wear material. Modulation of the actions of this cytokine has been explored as a potential treatment for particle-induced osteolysis. Etanercept, a soluble inhibitor of TNF-α, inhibits osteoclastic bone resorption in a bone wafer pit assay and cytokine production from titanium particle-stimulated macrophages.[16] Bone resorption and osteoclastogenesis were reduced in vivo when etanercept was given in a murine model of titanium particle-induced osteolysis. Gene delivery of a soluble inhibitor of TNF-α (sTNFR:FC) reduced titanium particle-induced osteolysis but not osteoclastogenesis in an athymic mouse calvarial model.[17] A clinical pilot study to evaluate the efficacy of etanercept in 20 patients with established periprosthetic osteolysis has been reported.[115] The outcome was progression of osteolysis assessed using a volumetric three-dimensional computerized tomography. In this study, there was no difference in progression of osteolysis between the etanercept and placebo-treated groups.[115] However, this was a pilot study and therefore not sufficiently powered to evaluate drug efficacy.

Antiinflammatory Cytokine Therapy

Periprosthetic osteolysis involves a cascade of proinflammatory mediators, cell-signaling mechanisms, and cellular elements. Antiinflammatory cytokine therapies are targeted at a broader spectrum of inflammatory events than more specific therapies, such as TNF-α blockade. This concept is important because many of the inflammatory pathways have complex and interrelated tracks, providing a redundancy in the inflammatory cascade. Two such antiinflammatory cytokines that have been examined as potential therapies for osteolysis are IL-4 and IL-10. In an in vitro model using titanium particles and cultures of monocytes and/or macrophages, addition of IL-4 or IL-10 suppressed TNF-α and IL-6 expression.[58] However, as yet no human trials have been reported.

RANK/RANKL Signaling Blockade

Inhibition of RANK-RANKL interactions has been effective in blocking bone loss in animal models of particle-induced osteolysis. AMG-162 is a human immunoglobulin G_2 monoclonal antibody with a very high affinity for human RANKL. The safety and bone antiresorptive effects of AMG-162 in postmenopausal women has been observed in Phase I and II clinical trials.[5,84] This drug may offer a future therapeutic or preventative agent for particle-induced osteolysis.

DISCUSSION

Implant failure as a consequence of particle-induced osteolysis is a persistent problem that limits the long-term success of hip arthroplasty. The cytokine pathways that modulate this process have been subject to much study in recent years. As a result of this work, the importance of the RANK-RANKL pathway in particle-induced osteolysis has become apparent. Numerous proinflammatory cytokines such as IL-1 and TNF-α are able to stimulate osteoclastogenesis via activation of NF-κB; however, these mediators do not represent the final common pathway to osteoclastogenesis. Central to regulation of this final process is RANKL, its receptor RANK, and the antagonist mol-

ecule OPG. Osteoclast precursors are unable to differentiate to mature osteoclasts in the absence of the RANK-RANKL interaction, despite the presence of particulate matter and TNF-α. Similarly, exogenous OPG is able to block particle- and TNF-α -stimulated osteoclastogenesis. Our recent work has shown another cytokine, IL-18, also may play a key role in modulating this process. This cytokine is able to inhibit particle-induced osteoclastogenesis and osteolysis in vitro. The mechanism of this inhibition would appear to be via increased OPG expression, although the source of this OPG has yet to be determined.

The RANK-RANKL-OPG pathway is not only fundamental to the process of osteoclastogenesis, it also is pivotal to function and development of the immune system.[3,69,129] RANKL secreted from activated T lymphocytes can directly modulate osteoclastogenesis and the activity of mature osteoclasts.[69] Mutant mice lacking T cells, however, still have normal bone cavities and tooth eruption,[129] and so T cells probably are not required for normal bone homeostasis. However, local inflammation in the bone, as a result of metastasis, infections, and fractures, or joint inflammation in arthritis attracts T cells, which may actively participate in bone remodeling though production of RANKL. Kong et al[69] reported lymph node organogenesis, T and B lymphocyte development, and osteoclast differentiation are all regulated by RANKL. In this way, the immune system and skeletal homeostasis are linked, which may account for osteopenia associated with conditions such as inflammatory arthritis.

Our improved understanding of the cytokines and receptors that influence particle-induced osteolysis has led to the trial of novel agents with the aim of pharmacologically modulating this process. Currently there are no approved pharmacologic therapies for particle-induced osteolysis. The potential therapies investigated so far include the bisphosphonates, anti-TNF-α, and anti-RANKL antibody. Although offering some promise, these compounds have not been shown to inhibit or reverse particle-induced osteolysis in a human population with sufficiently powered, randomized, controlled trials.

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