Periprosthetic osteolysis: genetics, mechanisms and potential therapeutic interventions

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Aseptic loosening and periprosthetic osteolysis occur as a result of the biological response to particulate wear debris and are one of the leading causes of arthroplasty failure. Periprosthetic osteolysis originates from chronic inflammatory responses triggered by implant-derived particulate debris, which cause recruitment of cells, including macrophages, fibroblasts, lymphocytes and osteoclasts. These cells secrete pro-inflammatory and osteoclastogenic cytokines, exacerbating the inflammatory response. In addition to their direct activation by phagocytosis, there are contributing autocrine and paracrine effects that create a complex milieu within the periprosthetic space, which ultimately governs the development of osteolysis. Chronic cell activation may upset the delicate balance between bone formation and bone resorption leading to periprosthetic osteolysis. This article summarizes the genetic mechanisms underlying periprosthetic loosening and identifies potential therapeutic agents.

L'ostéolyse aseptique associée à un descellement du pourtour d'une prothèse survient à la suite d'une réaction biologique à la présence de débris d'usure et constitue l'une des principales causes d'échec de l'arthroplastie. L'ostéolyse périprothétique résulte d'une réaction inflammatoire chronique, elle-même déclenchée par la présence de débris particulaires provenant de la prothèse, le tout stimulant le recrutement de cellules, telles que macrophages, fibroblastes, lymphocytes et ostéoclastes. À leur tour, ces cellules sécrètent des cytokines pro-inflammatoires ostéoclastiques et exacerbent la réponse inflammatoire. En plus de leur activation directe par la phagocytose, on assiste à des réactions autocrines et paracrines qui contribuent à créer un milieu complexe dans l'espace périprothétique et qui finalement modulent l'ostéolyse. L'activation cellulaire chronique peut perturber l'équilibre délicat entre ostéogenèse et résorption osseuse et entraîner de ce fait l'ostéolyse périprothétique. Cet article résume les mécanismes génétiques qui sous-tendent le descellement périprothétique et propose des agents thérapeutiques potentiels.

The major focus of research and development in arthroplasty over the past decade has been directed toward reduction of bearing surface wear and biologic reaction to periprosthetic debris. It is now well established that polyethylene (PE), metal, alumina and cement particles in the periprosthetic membrane cause activation of local cells.[1] Repeated phagocytosis of the wear debris particles, which are impervious to enzymatic destruction, results in activated inflammatory cells that secrete proinflammatory cytokines and proteolytic enzymes, leading to activation of a periprosthetic osteolytic cascade. The resulting bone loss or osteolysis may compromise implant fixation and ultimately result in component loosening. In this article, we summarize the genetic mechanisms underlying periprosthetic loosening and identify potential therapeutic agents.

Proinflammatory innate immune system cytokine gene activation

Macrophage activation by polymethylmethacrylate (PMMA) and polyethylene wear debris has been implicated as the principle pathophysiologic mechanism in particle-induced periprosthetic osteolysis.[1] Studies using retrieved tissue from failed metal-on-polyethylene articulations suggest heavy macrophage infiltration in the pseudomembrane around failed implants. Pearle and colleagues,[2] using

complementary DNA (cDNA) microarray profiling, studied peripheral blood mononuclear cells (PBMC) and monocytes response after stimulating them with PMMA and titanium particles of clinically relevant sizes. Polymethylmethacrylate induced high-level expression of proinflammatory cytokines, including tumour necrosis factor (TNF)–α, interleukin (IL)–1α, IL-1β, IL-6 and IL-8, eliciting a 6- to 12-fold increase in gene expression after 3 hours of culture in purified monocytes. The most highly induced gene was *PTGS2* (*COX2*), with a 30-fold increase in expression (Box 1). Stimulation of PBMC by PMMA for 3 or 6 hours resulted in a similar pattern of inflammatory cytokine expression to that observed in purified monocytes. In the case of PMMA-stimulated monocytes and PBMC, TNF-α, IL-6, IL-1α and IL-1β were coordinately expressed across all experiments based on hierarchical clustering of microarray data. Coexpressed with these inflammatory cytokines was a large group of chemokines, including monocyte inflammatory protein–3α (CCL20) and eotaxin 2 (CCL11). Significance analysis of microarray of all data from PMMA-stimulated cultures identified in creased expression of most of these same proinflammatory genes, with a false discovery rate of less than 5.

Fritz and colleagues[3] have shown that titanium particles result in enhanced IL-8 and monocyte chemotactic protein-1 (MCP-1) secretion as well as differential chemokine gene activation involving nuclear factor κβ (NF-κβ) activation (discussed in the subsequent RANK/RANKL section).

Activation of delayed adaptive immune response gene mediators

Studies investigating the pseudomembranes around metal-on-metal bearing prostheses, where metallic wear debris predominates, identified a potentially important pathogenic role of activated lymphocytes in the setting of cobalt chromium particulate load.[4,5] In vitro studies suggest various metals, particularly cobalt-chromium, may elicit a predominantly T helper 1 (Th-1) delayed-Type IV hypersensitivity response when exposed to lymphocytes.[6] Pearle and colleagues,[2] using cDNA microarray profiling, showed that in contrast to PMMA, titanium particles stimulated increased expression of T lymphocyte–derived cytokines, predominantly Th-1 cytokines, including IL-2, interferon-γ, IL-9, IL-13 and IL-22, in peripheral blood mononuclear cell cultures (Box 2). At 6 hours, multiple markers of T cell activation, including PRF1, GEM, IL2RA, CD8A, IL12RB2, TXK and CD69, were upregulated in PBMC in response to titanium stimulation but were only minimally affected by PMMA stimulation.

Interleukin-1 receptor antagonist

The proinflammatory cytokines IL-1α and IL-1β, known to mediate both in vivo and in vitro bone resorption, bind to IL-1 receptor type I (IL-1RI). They induce increased osteoclast formation and activation, effects that are mediated through RANKL,[7] as described in the subsequent section discussing RANKL. The IL-1R antagonist (IL-1Ra) also binds to IL-1RI and acts as a competitive antagonist to signal transduction. Gordon and colleagues[7] sought to study whether there is an association between osteolysis after total hip arthroplasty and common polymorphisms in the genes encoding the IL-1 family. Natural variation within the DNA coding sequence for a given gene in the population is termed polymorphism, and may alter an individual's susceptibility to disease by modulating gene transcriptional activation, altering the peptide product of the gene, or through other mechanisms. They reported that the less common c allele at single nucleotide polymorphism (SNP) rs419598 (IL-1RA + 2018), which encodes the anti-inflammatory cytokine IL-1Ra, was negatively associated with osteolysis. This was associated with increased IL-1 receptor antagonist mRNA expression in vitro. However, they did not report any information about the type of implants, number of participating surgeons and their experience, or details of polyethylene and PMMA used, which could also lead to premature failure associated with osteolysis, regardless of genotype.

Interleukin-6 gene promoter region

Gordon and colleagues[7] studied the association of polymorphisms encoding IL-6 genes with osteolysis following THA. They reported that a rare haplotype within the

Box 1. Mediators induced by polymethylmethacrylate

- Cytokines
  - TNF-α
  - IL-1α
  - IL-1β
  - IL-6
  - IL-8
- Genes
  - *PTGS2* (*COX2*)

IL = interleukin; TNF = tumour necrosis factor.

Box 2. Mediators induced by titanium

- T lymphocyte–derived cytokines
  - IL-2
  - IF-γ
  - IL-9
  - IL-13
  - IL-22
- Tyrosine kinase phosphorylation
- NF-κβ activity

IF = interferon; IL = interleukin; NF = nuclear factor.

gene for IL-6 (-174G/-572G/-597A, with a frequency of < 5% in their population) was positively associated with osteolysis (control group frequency 0.8%, osteolysis group frequency 2.4%; $p = 0.020$). Interestingly, Malik and colleagues[8] found no statistically significant relationship between aseptic loosening and IL6–174 SNPs.

Wnt signalling

The wnt glycoproteins are a group of recently identified secreted glycoproteins that play an important regulatory role in the differentiation, growth and apoptosis of many embryologic and adult cell types, including the osteoblast. Secreted Frizzled related protein-3 (sFRP3) is a glycoprotein that antagonizes the signalling of wnt ligands through the frizzled membrane-bound receptors. The gene encoding sFRP3 is located on the long arm of chromosome 2 (2q32.1) and its approved symbol is *FRZB*. Gordon and colleagues[7] examined whether variation in *FRZB* is associated with the development of osteolysis after total hip arthroplasty. They showed that the carriage rate of the FRZB 200Trp allele was 32% lower in the osteolysis patients versus controls ($p = 0.041$). After adjusting for the effects of independent risk factors using multiple logistic regression analysis, the odds ratio (OR) for osteolysis associated with carriage of the FRZB 200Trp allele was 0.62 ($p = 0.049$).

TNF gene promoter

Wilkinson and colleagues[9] studied whether natural variance within the promoter region of the TNF gene contributes to the risk of osteolysis after cemented THA. They included 481 white patients (214 with failed v. 267 with intact implants) a mean of 11.7 (standard deviation [SD] 4) years after cemented THA. The allele frequency and OR for carriage of the SNP guanine to adenine transition at position –238 in patients with osteolysis was approximately twice that in patients with successful implants and the background population. The relationship between carriage of the SNP guanine to adenine transition at position –238 and osteolysis was strongest in those with both femoral and pelvic osteolysis.

RANKL/RANK and osteoprotegerin OPG system

The receptor activator of NF-κβ (RANK) is a novel type I transmembrane receptor of the TNF receptor superfamily (TNFRSF) that is ubiquitously expressed in human tissues, but is particularly apparent on the cell surface of osteoclasts and their precursors.[10] Binding of the receptor activator of NF-κβ ligand (RANKL) to its cognate receptor RANK induces NF-κβ signalling, resulting in NF-κβ translocation to the nucleus with a subsequent increase in Re1B levels, in turn, stimulating osteoclastic differentiation (Fig. 1). Mice deleted of the p50/p52 NF-κβ heterodimer (TNF $^{-/-}$) develop osteopetrosis, a condition in which the animals lack osteoclasts. Clohisy and colleagues[11] reported that exposure of osteoclast precursors, in the form of colony stimulating factor-1 (CSF-1)–dependent murine bone marrow macrophages, to PMMA particles prompted nuclear translocation and activation of NF-κβ. A soluble, competitive inhibitor of TNF (huTNFr:Fc) dampened particle-directed NF-κβ activation, and this response was also abrogated in TNF$^{-/-}$ osteoclast precursors.

Osteoprotegerin (OPG) a decoy receptor, expressed by osteoblasts, binds with RANKL, preventing RANK signalling and thus inhibiting osteoclastogenesis (Fig. 1). Ren and colleagues[10] introduced ultra-high molecular-weight PE (UHMWPE) debris into established air pouches on RANK$^{-/-}$ mice, followed by implantation of calvaria bone from syngeneic littermates. They showed that UHMWPE particles induced similar pouch tissue inflammation and increased gene expression of RANKL, TNF-α and IL-1β in RANK$^{-/-}$ mice. More importantly, the UHMWPE particle-induced osteoclastic bone resorption that developed in RANK$^{+/+}$ mice was not observed in RANK$^{-/-}$ mice. This suggested that RANK is the sole receptor of RANKL and is essential for the development of UHMWPE particle-induced osteoclastic bone resorption. These results were in agreement with those of Schwarz and colleagues,[12] who reported that treatment with antagonist RANK:Fc IgG successfully prevented titanium debris-induced inflammatory osteolysis in a mouse calvaria model, yielding results that were statistically equivalent to data obtained with titanium-treated RANK$^{-/-}$ mice.

Atkins and colleagues[13] developed a novel 3-dimentional (3-D) culture system to study the effect of PE particles on human osteoblastic cells. This involved culturing osteoblasts in a type 1 collagen gel, throughout which PE particles were dispersed, effectively juxtaposing the otherwise hydrophobic PE particles with cells over prolonged periods of up to 4 weeks. Normal human bone-derived cells (NHBCs) responded to PE particles by increasing the mRNA expression of several genes associated with osteoclast formation and activity (RANKL, IL-8 and MCSF) and decreased the expression of the osteoclast antagonist, OPG. Polyethylene also appeared to induce a switch in the RUNX2 control of gene expression from that of promoting

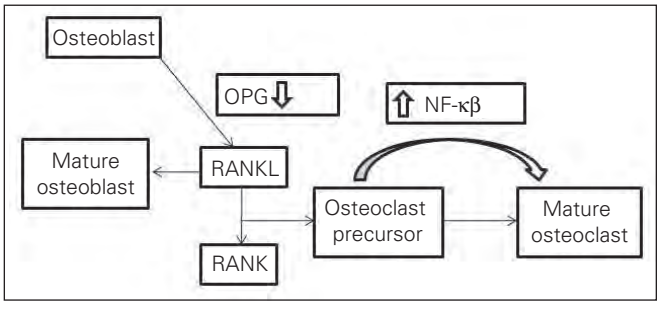

Fig. 1. Activation of the RANKL system.

matrix production (type 1 collagen) to inducing the expression of pro-osteoclastogenic genes, suggesting that PE particles switch mature osteoblastic cells from an anabolic to a more catabolic phenotype.

Bylski and colleagues[14] used THP-1 macrophage-like cells to study the effect of commercially produced ceramic and titanium particles with different diameters and concentrations on the mRNA expression of RANK, RANKL, OPG and TNF-α. They found that alumina ceramic particles, regardless of particle size, caused only slight upregulations of RANK, TNF-α and OPG mRNA whose levels were significantly lower as compared with titanium particles (all $p < 0.05$). The continuous increasing tendency to time and particle-dependent mRNA expression of all parameters stimulated by titanium particles was not found after stimulation with ceramic materials.

Baumann and colleagues,[15] using the human monocytic leukemic cell line (THP-1) showed both PE and prosthesis-derived titanium aluminum vanadium (TiAlV) particles induced a significant release of TNF-α after 6 hours of exposure (17-fold for TiAlV and 15-fold for PE, all $p < 0.05$) and a significant upregulation of RANK mRNA (3.5-fold increase for TiAlV $p = 0.040$ and 3-fold increase for PE, $p = 0.040$). They found that OPG mRNA expression was transiently upregulated after exposure to PE and TiAlV particles. Moreover, these effects were dependent on particle dose. The RANKL mRNA expression was not detectable in their model. Granchi and colleagues[16] incubated human MG63 osteoblast-like cells with 4 radio-opaque cement extracts, including Sulfix-60, CMW1, CMW2 and CMW3, and using reverse transcription polymerase chain reaction (RT-PCR) found that all increased the OPG-L/OPG ratio more than that for IL-1β, the positive control.

Nuclear factor–κβ is the primary transcriptional activator and regulator of numerous proinflammatory cytokine and chemokine genes, including IL-8 and MCP-1, suggesting a mechanism of cellular recruitment to the periprosthetic region.[3] More specifically, PMMA and titanium-stimulated MCP-1 secretion in particulate challenged fibroblasts, and MCP-1 and macrophage inflammatory protein-1 α (MIP-1α) gene expression was observed in PMMA and Ti6Al4V alloy-exposed macrophages. Fritz and colleagues[3] illustrated that human bone marrow–derived osteoblast cells responded to particle stimulation with varied kinetics of gene activation and differential induction of IL-8 and MCP-1 mRNA synthesis, suggesting a potential donor-dependent osteoblast response to titanium particle challenge. Interleukin-8 chemokine expression was about 4-fold greater than MCP-1 in titanium-stimulated bone marrow–derived osteoblasts. In addition, IL-8 but not MCP-1, gene regulation was upregulated in all 5 patient samples in response to titanium particle challenge. These findings suggest an attractive hypothesis for why patients with implants that generate similar quantities of particulate wear debris may exhibit differing patterns and magnitudes of osteolysis. Perhaps differential gene activation by particulate wear debris in cell populations of the bone–prosthesis microenvironment can account for this apparent disparity in the development of periprosthetic osteolysis.

Nuclear factor of activated T cells

Nuclear factor of activated T cells (NFAT) is another transcription factor that functions downstream of RANKL in osteoclast differentiation. It has been shown that RANKL activates the TNF receptor–associated factor 6 (TRAF-6) and c-Fos pathways, leading to autoamplification of NFATc1 to induce osteoclast differentiation.[17] Liu and colleagues[18] investigated the role of NFATc1 in the regulation of osteoclast differentiation from bone marrow macrophages (BMMs) stimulated with titanium particles. They showed that titanium particles could stimulate BMMs to produce proinflammatory cytokines (TNF-α, IL-1β and IL-6) and differentiate into multinucleated osteoclasts in the presence of RANKL. They also showed that NFATc1 was expressed in BMMs and multinucleated cells cultured with titanium particles and RANKL. Furthermore, inactivation of NFATc1 by the peptide 11R-VIVIT potently impeded the titanium particle–induced osteoclastogenesis.

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF), the most potent angiogenic growth factor, is produced by many cells, including macrophages, lymphocytes and osteoblasts. Ren and colleagues[19] introduced UHMWPE particles into established air pouches on BALB/c mice, followed by implantation of calvaria bone from syngeneic littermates. Mice were injected with either recombinant VEGF or VEGF inhibitor (VEGF R2/Fc chimera). The UHMWPE stimulation significantly increased VEGF gene expression which in turn was markedly reduced by VEGF inhibitor treatment. Pouch membrane macrophages were the predominant cells expressing VEGF, and there was a positive association between tissue inflammation status and the levels of VEGF gene activity. Both RANK and RANKL gene transcripts were significantly increased by UHMWPE stimulation, which was subsequently reduced by VEGF inhibitor ($p < 0.05$). In addition, VEGF treatment was shown to increase TRAP+ cells in pouches either with or without UHMWPE particle stimulation, and the fact that VEGF inhibitor treatment significantly reduced the number of TRAP+ cells in UHMWPE-containing pouches suggests that VEGF exerts a critical role in the development of osteoclastogenesis, which may be mediated through the RANK–RANKL pathway.

Mitogen-activated protein kinases

Mitogen-activated protein (MAP) kinases are proline-directed serine/threonine kinases that are important in cell growth, differentiation and apoptosis. They are regulated by osteoclastogenic factors of the TNF and RANKL family and are essential for osteoclast differentiation and activation. The c-Jun N-terminal kinases (JNKs) are a subfamily of MAP kinases that play an integral role in both acute and chronic inflammation. Yamanaka and colleagues[20] demonstrated that PMMA particles activate the JNK pathway in wild-type C57Bl/6 and TLR4-null (endotoxin resistant) osteoclast precursors. Furthermore, this activation was significantly blocked in a dose-dependent and reversible manner by the JNK inhibitor SP600125.

Matrix metalloproteinases

Matrix metallopreoteinases (MMPs) are a family of more than 20 homologous proteolytic enzymes, which can degrade almost all components of the extracellular matrix. The proteolytic activities of MMPs are controlled at the level of activation from their precursors and by inhibition of already activated MMPs by their endogenous inhibitors, α-2-macroglobulins and tissue inhibitors of metalloproteinases (TIMPs). Takei and colleagues[21] analyzed periprosthetic tissues from 18 patients to study the mRNA expression pattern of 16 different types of MMPs. They showed highly elevated expression of MMP-1, -9, -10, -12 and -13; moderate expression of MMP-2, -7, -8, -11, MT1-MMP (MMP-14), MT2-MMP (MMP-15), MT3-MMP (MMP-16), MT4-MMP (MMP-17) and -19; lower expression of MMP-3 and little significance of MMP-20. Sasaki and colleagues,[22] using periprosthetic tissues from 15 aseptic loose hip joints at surgery, showed that upregulation of mRNA TIMP-1, -2 and -3 was significantly higher ($p < 0.05$) and expression of TIMP-4 was significanly lower ($p < 0.01$) than that in the control group. Nawrocki and colleagues[23] used immunohistochemistry to identify the cells responsible for the synthesis of MMPs. They detected gelatinase A mRNAs in fibroblasts and showed that the protein was preferentially localized at the periphery of multinucleated giant cells, leading them to hypothesize that fibroblasts cooperate with macrophages and multinucleated giant cells, leading to osteolysis.

Genetic variation may determine individual responses in terms of susceptibility to osteolysis. In a case–control study, Malik and colleagues[8] showed that an SNP of MMP1 was highly associated with aseptic loosening failure when compared with controls. Their cases included 91 patients with early aseptic loosening and 71 patients with microbiological evidence of deep infection, whereas controls included 150 patients with total hip replacements clinically asymptomatic for more than 10 years and showing no radiographic features of aseptic loosening. The frequencies of the C allele (OR 3.27, 95% confidence interval [CI] 2.21–4.83, $p = 0.001$) and C/C genotype ($p = 0.001$) for the MMP1 SNP were highly associated with aseptic failure as compared with controls. This MMP1 SNP exists within a promoter region of the gene and, as such, may have a direct effect on the level of gene expression.

Substance P

Substance P (SP)–immunopositive axons are distributed in the periosteum, bone marrow and sensory nerve fibres, and the release of this neuropeptide leads to neurogenic inflammation. Ahmad and colleagues[24] demonstrated rare SP and calcitonin gene–related peptide (CGRP) immunoreactive nerve fibres in the interface membrane of patients with failed total hip arthroplasties. Substance P belongs to the tachykinin family and interacts with the neurokinin$_1$ receptor (NK_1R). Wedemeyer and colleagues[25] used the murine calvarial osteolysis model based on UHMWPE particles in 14 wild-type mice (C57BL/J6) and 14 SP-deficient mice. Groups 1 (C57BL/J6) and 3 (SP-knockout) received sham surgery, and groups 2 (C57BL/J6) and 4 (SP-knockout) were treated with PE particles. The UHMWPE particle–treated SP-deficient mice showed significantly reduced osteolysis compared with wild-type mice, as confirmed by histomorphometry ($p < 0.001$) and microcomputed tomography ($p < 0.035$). Osteoclast numbers were significantly reduced in groups 3 and 4 compared with groups 1 and 2 ($p < 0.001$). Interestingly, SP-deficient mice (group 3) showed a significantly increased absolute bone mass compared with wild-type mice (group 1; $p = 0.020$), suggesting that the local neurogenic microenvironment is a significant factor in the remodelling of loose implants. Therefore neurogenic inflammation mediated via the nervous system may play an important role in the UHMWPE particle–induced osteolysis.

Mast cell proteases

Mast cells have been implicated in bone resorption adjacent to failing implants. Qiu and colleagues[26] studied the interface membrane of 32 aseptically loosened total hip implants, of which 28 had mast cells of the MC_{TC} type. These cells express tryptase, chymase, cathepsin G and mast cell carboxypeptidase, and they have been shown to actively produce chymase mRNA.

Cell substrate interactions

Shen and colleagues[27] used immunohistochemical, histochemical and in situ hybridization techniques to analyze the phenotype of cells in human peri-implant tissues from 12 patients undergoing revision hip arthroplasty for aseptic loosening. A marker expressed by multiple macrophage lineage cell types, CD68 protein, was detected in mononucleated and

multinucleated cells associated with PE particles and the bone surface. Cathepsin K and tartarate-resistant acid phosphatase were expressed highly in both mononucleated and multinucleated cells associated with the bone surface. Levels of expression were much lower in cells associated with PE particles. High levels of β_3 integrin protein were detected in cells in contact with bone. Multinucleated cells associated with PE particles exhibited faint positive staining. Calcitonin receptor mRNA expression was detected solely in multinucleated cells present in resorption lacunae on the bone surface and was absent in cells associated with PE particles. Osteoclasts and foreign body giant cells are derived from a common hematopoietic precursor, and Shen and colleagues[27] hypothesized that, in addition to the role of cytokines and growth factors, the substrate with which these cells interact plays a critical role in their differential phenotypic and functional properties.

Alternative macrophage activation and impaired osteogenesis

Evidence in favour of an important role for proinflammatory cytokines in osteolysis include the observations that wear particles can induce the production of these cytokines in both cultured macrophage lineage cells[28,29] and murine models of osteolysis[30,31] and that blockade of proinflammatory signalling can ameliorate disease in these animal models.[31–33] Koulouvaris and colleagues[34] showed that wear debris–induced osteolysis is also characterized by an alternative, nonproinflammatory macrophage activation pathway, leading to perturbations in the periprosthetic cytokine and chemokine milieu favouring osteoclast precursor cell recruitment and maturation. Using real-time PCR (RT-PCR) analysis of periprosthetic soft tissue from patients with osteolysis, the authors detected elevated levels of expression of alternative macrophage activation markers (CHIT1, CCL18), chemokines (IL-8, MIP1α) and markers of osteoclast precursor cell differentiation and multinucleation (Cathepsin K, TRAP, DC-STAMP) relative to osteoarthritis controls. Furthermore, they showed that this proresorptive imbalance is exacerbated by wear debris–driven defects in osteogenesis with reduced expression levels of osteogenic signalling components BMP4 and FGF18.

Kwon and colleagues[35] studied the influence of titanium particles on osteoblastic gene expression profile using Northern blot analysis and RNase protection assay. The proliferation study demonstrated that the inhibition of osteoblast proliferation is positively correlated with increased titanium concentration and incubation time. The gene expression assays indicated that type 1 collagen and fibronectin expression is perturbed by incubation with titanium particles and is related to the period for which the osteoblasts are incubated with the particles. Furthermore, apoptotic gene expression was shown to be activated in long-term exposure.

Macrophage cell surface receptors — complement receptor 3 and scavenger receptor A

Rakshit and colleagues[36] showed that activation of signalling pathways in macrophages by PMMA and titanium wear particles is mediated by the cell surface receptors complement receptor 3 (CR3) and scavenger receptor A (SRA). They demonstrated that several serum proteins, including known opsonins such as C3bi and fibronectin, adhered to PMMA but not to titanium and that serum was required for proinflammatory signalling induced by PMMA but not by titanium. Blocking CR3 specifically inhibited phagocytosis of PMMA by macrophages, whereas blocking SRA specifically inhibited titanium uptake. Furthermore, the authors reported direct involvement of CR3 and SRA in cell-particle interaction by expression of these receptors in nonphagocytic HEK293 human embryonic kidney fibroblasts. They reported that CR3 specifically induced cell binding to PMMA particles and adhesion to PMMA-coated plates, whereas SRA specifically induced binding to titanium particles and titanium-coated plates.

Protein tyrosine kinases

Phagocytosis of particulate biomaterials activates protein tyrosine kinase (PTK) pathways, which result in altered gene expression via the activation of nuclear transcription factors. Vermes and colleagues[37] showed that the exposure of osteoblast-like osteosarcoma cells and bone marrow–derived primary osteoblasts to commercially pure titanium and UHMWPE particles of phagocytosable small size (< 3 μm in diameter) and nonphagocytosable large size (> 20 μm) resulted in a marked decrease in the steady state mRNA levels of procollagen α1(1) and procollagen α1(111). This particle effect in osteoblasts was gene-specific because particles had little or no effect on several osteoblast-specific genes, including osteonectin and osteocalcin. Interestingly, large particles that could not be phagocytosed also downregulated collagen gene expression, suggesting that an initial contact between cells and particles can generate gene responsive signals, independent of the phagocytosis process. With respect to this signalling, they showed that comercially pure titanium particles rapidly increased protein tyrosine phosphorylation and NFκB binding activity before the phagocytosis of particles. Furthermore, they demonstrated that PTK inhibitors, such as genistein and NFκB inhibitor pyrrolidine dithiocarbamate significantly reduced the suppressive effect of comercially pure titanium on collagen gene expression. Thus particles can initiate gene responses in osteoblasts independently of phagocytosis, although

a long-term cell response appears to require particle phagocytosis.

Obesity gene and leptin protein

The obesity gene is known to affect both bone mass and body mass by its 16kDa encoded leptin protein, which affects bone metabolism. Von Knoch and colleagues,[38] using the calvarial osteolysis model implanted PE particles onto calvaria in 7 wild-type C57BL/6J mice and in 7 obese (ob/ob) C57BL/6J-Lep^{ob} mice to stimulate particle-related osteolysis. The average osteoclast number per millimetre of total bone perimeter was 8 (SD 3.464) in wild-type animals with particles and 2.857 (SD 1.676) in ob/ob animals with particles ($p < 0.001$). Bone resorption was 1.895 (SD 0.713) mm/mm^2 in wild-type animals with particles and 1.265 (SD 0.494) mm/mm^2 in ob/ob animals with particles ($p = 0.044$), suggesting that obesity may have a protective role against particle-induced bone resorption — similar to obesity and osteoporosis.

Potential therapeutic interventions

Understanding the mechanisms by which osteoclasts resorb bone and the cytokines that regulate their differentiation and activity provides mechanism-based potential therapeutic targets to prevent inflammatory bone loss. Several investigators have taken advantage of a huge array of genetically defined mice to examine molecular genetics of wear debris–mediated osteolysis in vivo. These include TNFR (–/–) and NFκB (–/–),[12] Cox-1 (–/–) and Cox-2 (–/–),[39] TNF-Tg,[40] RANK (–/–).[41] IL-6 (–/–) and IL-1R (–/–) mice. In terms of drug and gene therapy, several substances have been evaluated: alendronate and pentoxifylline,[30] etanercept celecoxib,[39] RANK:Fc,[41] OPG,[42,43] TNFR:Fc,[40] viral IL (vIL)–10[44] anti-IL-6 and anti-IL-1R. These have elucidated the biological hierarchy in which RANK blockade is clearly the safest and most effective means to prevent and ameliorate wear debris–induced osteolysis.

Shanbhag and colleagues[45] reported that oral alendronate therapy inhibited osteolysis induced by a mixture of UHMWPE, titanium alloy and cobalt-chromium alloy in a canine total hip replacement model. Alendronate was shown to inhibit wear debris–mediated osteolysis for the 24-week duration of the study, but histologic and biochemical data showed that periprosthetic inflammation was not reduced. A novel synthetic bisphosphonate, TRK-530, has been shown to reduce bone resorption in rats with adjuvant arthritis by inhibiting the IL-1–like activity of resident peritoneal macrophages and by decreasing the bone marrow levels of cytokine-induced neutrophil chemoattractant-1 and TNF-α. Iwase and colleagues[46] randomly assigned 40 Wistar rats to 2 groups. In each rat, a K-wire was inserted into the femur and high-density PE particles were continuously infused into the knee joint. Every second day thereafter, the animals were subcutaneously injected with saline (control group) or 1 mg/kg of TRK-530, and were sacrificed at 4 or 8 weeks after surgery. Radiographs obtained at the time of sacrifice were evaluated for periprosthetic osteolysis. The authors also examined the thickness of the reactive membrane and the number of osteoclast-like cells around the K-wire. In addition, they examined the expression of genes for bone-resorbing cytokines in the reactive membrane. Radiographic periimplant osteolysis was more frequent in the control than the TRK-530 group at each assessment ($p < 0.01$). The interfacial membrane was significantly thinner in the TRK-530 than the control group ($p < 0.01$), and the average number of osteoclast-like cells around the K-wire was significantly lower in the TRK-530 than the control group ($p < 0.01$). In addition, the expression of IL-1α messenger RNA (IL-1αmRNA) and TNF-αmRNA was suppressed in the TRK-530 group at each assessment. Because statins, as HMG-CoA reductase inhibitors, also target the mevalonate pathway, they have been considered possible drugs for osteolysis. Simvastatin prevents wear debris–induced osteolysis in the murine calvarial model.[47]

Gene therapy offers a novel approach to treat chronic inflammation that avoids most of the weaknesses of the conventional therapies. By directly delivering the genes encoding for anti-inflammatory cytokine proteins to the local sites, gene transfers may provide a relatively sustained therapeutic effect to match the persistence of the inflammation and reduce side effects caused by systemic administration.[48] Yang and colleagues[48] evaluated the effects of anti-inflammatory cytokine gene transfer on osteolysis. A section of bone was surgically implanted into an air pouch established on a syngeneic recipient mouse. Inflammation was provoked by introduction of UHMWPE particles into the pouch, and retroviruses encoding for IL-1 receptor antagonist (hIL-Ra), vIL-10 or LacZ genes were injected. Pouch fluid and tissue were harvested 7 days later for histological and molecular analyses. Results indicated that in vivo therapeutic gene transfer of human IL-1Ra or vIL-10 dramatically abolished the characteristic markers of UHMWPE particle–induced local inflammation. Findings included reduced pouch membrane thickness, decreased inflammatory cellular infiltration and diminished expression and secretion of proinflammatory cytokines (IL-1 and TNF). Furthermore, the incidence of bone pit erosions and bone collagen degradation was dramatically reduced. Carmody and colleagues[44] used replication-defective adenovirus vectors expressing vIL-10 (AdvIL-10) or LacZ (AdLacZ) target genes to transduce fibroblast-like synoviocytes (FLS) in vitro to evaluate wear debris–induced osteolysis in a mouse calvaria model. In the presence of AdLacZ-infected FLS, titanium particle–stimulated macrophages exhibited a marked increase in secretion of TNFα (6.5-fold), IL-6 (13-fold) and IL-1 (5-fold). Coculture with AdvIL-10–transduced FLS suppressed cytokine

secretion to basal levels, whereas addition of an anti-IL-10 neutralizing antibody completely blocked this effect. The vIL-10–transduced FLS also inhibited osteoclastogenesis 10-fold in an anti-IL-10–sensitive manner. In vivo, titanium implantation resulted in a 2-fold increase in osteoclasts ($p < 0.05$) and a 2-fold increase in sagittal suture area ($p < 0.05$). This increase over control levels was completely blocked in mice receiving intraperitoneal injections of AdvIL-10, all of whom had measurable serum vIL-10 levels for the duration of the experiment.

Yang and colleagues[48] evaluated retrovirus-mediated gene therapy using a novel xenograft-based animal model. Severe combined immunodeficient (SCID) mice do not reject xenografts because of a lack of functional T and B lymphocytes, which provides a means to study gene transfer effects in vivo on human periprosthetic tissue. The authors showed that human periprosthetic tissues were well accepted in SCID mice for up to 30 days. Strong expression of IL-1, TNF and IL-6 was detected in the xenografts using immunohistochemical stains. Histological analysis revealed that IL-1 receptor antagonist gene modification significantly decreased the total number of inflammatory cells ($p < 0.01$) in engrafted human tissue containing implant wear debris. Real-time RT-PCR and immunohistochemical staining showed declining expression levels of IL-1 and TNF following IL-1 receptor antagonist gene transfer in comparison with LacZ-transduced or virus-free controls. A limitation of retroviral vector transduction is that they only infect dividing cells and have the tendency to mutate. Tumorigenicity has also been reported in clinical trials using retroviral vectors.

Ex vivo gene therapy is an approach in which cells are removed from the patient, transduced with the target gene in vitro and are then reintroduced into the patient. Goater and colleagues[42] explored the potential of ex vivo OPG gene therapy for aseptic loosening by evaluating the efficacy of stably transfected FLS expressing OPG in preventing wear debris–induced osteoclastogenesis in a mouse calvaria model. Although the stably transfected fibroblasts produced small amounts of OPG (0.3 ng/mL/72 h/10^6 cells), the local secretion of this protein was very effective in blocking osteoclastic bone resorption in vitro and titanium-induced osteoclastogenesis in vivo. However, these effects did not last beyond 3 days, and in vitro OPG expression was undetectable in vivo. The authors then went on to develop an in vivo gene therapy model using recombinant adeno-associated virus vector (rAAV)–mediated OPG gene transfer[43] in a mouse calvarial model. Viral vector-mediated gene transfer provides a novel approach to delivering the anti-inflammatory genes to the site of disease to produce therapeutic proteins in a persistent and localized manner. A single intramuscular injection of the vector was administered before the introduction of the wear debris, which resulted in detectable transduction of myocytes at the injection site and a significant increase in expression of serum OPG levels by the second day ($p < 0.05$). Maximal concentrations were obtained on day 6 and then levelled off throughout the observation period. In contrast, serum OPG could not be detected in the sham-treated, uninfected titanium-stimulated or rAAV-LacZ-infected mice. In the control mice, titanium implantation resulted in a 3-fold increase in the mean number of osteoclasts adjacent to the sagittal suture as well as a 2-fold increase in the mean area of soft tissue in the sagittal suture compared with sham-treated mice. In contrast, osteoclast numbers remained at basal levels, and the area of soft tissue in the sagittal suture was markedly reduced in titanium-implanted animals that received an rAAV coexpressing OPG (rAAV–OPG–IRES–EGFP; internal ribosome entry sequence–enhanced green fluorescent protein treatments), demonstrating a complete a complete inhibition of osteolysis in response to titanium particles.[43]

Yang and colleagues[48] evaluated AAV-mediated OPG gene transfer to protect against particulate UHMWPE–induced osteolysis in a murine air pouch model of bone resorption. Adeno-associated virus vectors have shown some distinct advantages over other viral vectors; they can infect both dividing and nondividing host cells and provoke a very limited host immune response, unlike the adenoviral vectors. Moreover, AAV vectors also have better long-term transgene expression. Yang and colleagues[48] implanted bone tissue into established pouches in BALB/c mice, followed by the introduction of UHMWPE particles. The viruses encoding the human OPG gene (rAAV-hOPG) or the β-galactosidase marker gene (rAAV-LacZ) were injected into the air pouches, and the tissue was harvested 7 days after viral infection for histologic and molecular analyses. Real-time PCR indicated significant diminishment of mRNA expression of osteoclast markers in OPG-transduced pouches compared with rAAV-LacZ-transduced pouches. The transduction and expression of OPG also markedly decreased the gene copies of the biologic receptor activator of NFκβ. Computerized image analysis revealed that expression of OPG significantly protected against bone collagen loss.

An alternative way to suppress overexpression of TNF-α is antisense technology, including antisense oligodeoxynucleotide, ribozyme and RNA interference. Dong and colleagues[49] used a single subcutaneous dose of a verified antisense oligodeoxynucleotide (ASO) targeting to mouse TNF-αmRNA with the murine calvaria osteolysis model in C57BL/J6 mice. They reported an average bone resorption of 0.347 (SD 0.09) mm^2 in mice with Co-Cr-Mo particle implantation, which decreased to 0.123 (SD 0.05) mm^2 and 0.052 (SD 0.02) mm^2 after low- and high-dose ASO treatment, respectively. The number of osteoclasts in animal calvaria treated with ASO was reduced compared with that of untreated animals, and the quantification results indicated that about 90% of osteoclastogenesis was suppressed by ASO. Furthermore, with the addition

of TNF-α, osteoclastogenesis was re-established. Other antisense strategies, such as RNA interference and ribozyme, can also be used with this concept.

Conclusion

Understanding the responsible molecular and cellular pathways is critical to rational identification of treatment strategies for prevention and treatment of periprosthetic osteolysis. Further research in the cellular and molecular pathogenesis of osteolysis is needed to identify molecular candidates as potential therapeutic targets. Future studies designed to assess safety, efficacy and biomechanical analysis in large animal models will provide answers to this clinical problem.

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