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Review

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Novel biological strategies for treatment of wear particle-induced periprosthetic osteolysis of orthopaedic implants for joint replacement

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Wear particles and by-products from joint replacements and other orthopaedic implants may result in a local chronic inflammatory and foreign body reaction. This may lead to persistent synovitis resulting in joint pain and swelling, periprosthetic osteolysis, implant loosening and pathologic fracture. Strategies to modulate the adverse effects of wear debris may improve the function and longevity of joint replacements and other orthopaedic implants, potentially delaying or avoiding complex revision surgical procedures. Three novel biological strategies to mitigate the chronic inflammatory reaction to orthopaedic wear particles are reported. These include (i) interference with systemic macrophage trafficking to the local implant site, (ii) modulation of macrophages from an M1 (pro-inflammatory) to an M2 (anti-inflammatory, pro-tissue healing) phenotype in the periprosthetic tissues, and (iii) local inhibition of the transcription factor nuclear factor kappa B (NF-κB) by delivery of an NF-κB decoy oligodeoxynucleotide, thereby interfering with the production of pro-inflammatory mediators. These three approaches have been shown to be viable strategies for mitigating the undesirable effects of wear particles in preclinical studies. Targeted local delivery of specific biologics may potentially extend the lifetime of orthopaedic implants.

1. Introduction

Total joint replacement (TJR) of the lower extremity is a highly successful surgical procedure for alleviating pain, and improving ambulation and function for patients with end stage arthritis of the hip and knee. However, all TJR implants undergo wear of the bearing surfaces, producing particulates and other by-products of the different materials used in the surgical reconstruction [1]. These particulates, in sufficient amounts, may lead to the destruction of bone in the implant bed (periprosthetic osteolysis) jeopardizing the longevity of the implant. This may necessitate further surgery to replace worn parts, complex revision surgery to excise loose implants and re-implant new ones, or the fixation of pathologic fractures dues to bone degradation.

Debris from polymers such as polyethylene (PE) and polymethylmethacrylate (PMMA), metals and ceramics are capable of inciting an adverse tissue reaction, which is orchestrated by cells of the monocyte/macrophage lineage.

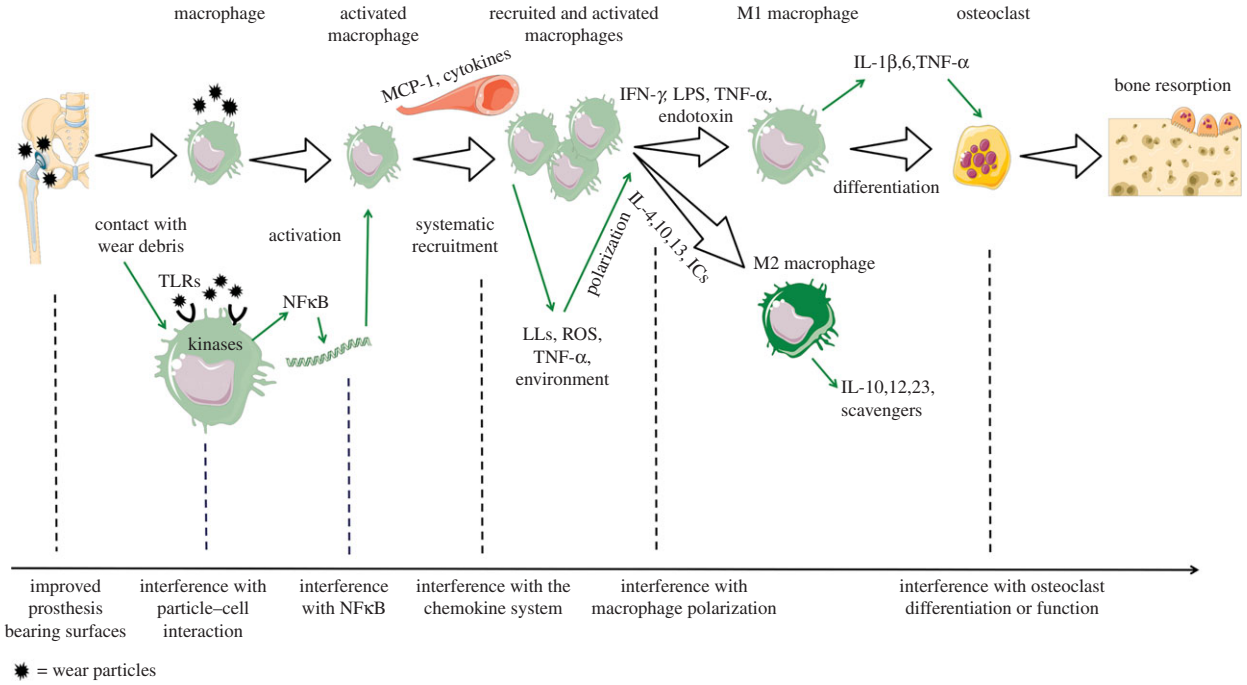

Figure 1. Biological strategies for treatment of wear particle-induced periprosthetic osteolysis. This figure outlines some potential biological approaches to preventing and treating periprosthetic osteolysis owing to wear particles from orthopaedic implants.

Particles and their by-products are phagocytosed by macrophages and other local cells, which become activated [2–5]. This results in the release of pro-inflammatory substances such as cytokines, chemokines, prostanoids, degradative enzymes, reactive oxygen species and other factors [6–12]. This chronic inflammatory scenario leads to upregulation of pathways leading to bone destruction, and downregulation of bone formation [13–15]. Thus, the normal homoeostatic mechanisms that lead to continued successful coexistence between the implant and the surrounding musculoskeletal tissues is perturbed [16,17]. These events may lead to painful synovitis and impaired function including restriction of range of motion, instability and loosening of the implant, and pathologic fracture of the surrounding bone.

There are no pathognomonic laboratory tests to diagnose ongoing periprosthetic osteolysis. Surgeons rely on the clinical presentation and periodic serial radiographic imaging studies, which may demonstrate evidence of progressive wear of the bearing surfaces, osteolysis, loosening of the prosthesis or fracture [18,19]. In some cases, these events can be detected when the prosthesis is still salvageable, that is, still well fixed and asymptomatic, such as when early wear and minimal bone destruction have occurred. Although weight loss and activity restriction may theoretically slow down the wear rate, these options have proved to be impractical. To date, no biological approaches to curtailing the chronic inflammatory reaction associated with wear particles have proved successful clinically, owing to the plethora of inflammatory mediators that are upregulated by particles, redundancy of inflammatory pathways and potential adverse effects of systemic treatments [20,21].

Although the chronic inflammatory reaction to implant degradation products is primarily a local phenomenon, remote inflammatory and mesenchymal cells migrate to the anatomical location where the particles are generated [22–25]. This cellular trafficking occurs along chemokine or chemorepellent gradients produced by local cells exposed to the by-products of wear. Systemic treatments attempting to limit one pro-inflammatory cytokine or induce osteoclast apoptosis have been unsuccessful in mitigating osteolysis. *In vitro* and *in vivo* models that use some novel therapies to modulate particle-induced inflammation, for example gene therapy, risk potential adverse events [26].

Our group has approached the problem of osteolysis due to wear particles as a local biological phenomenon that could theoretically be modulated by local rather than systemic treatment. Three experimental approaches have been taken to potentially mitigate the adverse biological sequela of particle disease. These include (i) interfering with ongoing migration of monocyte/macrophages to the implant site by inhibiting the chemokine–receptor axis [23,27], (ii) altering the functional activities of local macrophages by converting pro-inflammatory M1 (classically activated pro-inflammatory) macrophages to an anti-inflammatory pro-tissue healing M2 phenotype [28–30], and (iii) modulating the production and release of pro-inflammatory chemokines, cytokines and other potentially harmful factors by inhibiting the key transcription factor nuclear factor kappa B (NF-κB) ([31–33]; figure 1).

2. Interfering with ongoing migration of monocyte/macrophages to the implant site by modulating the chemokine–ligand–receptor system

In the process of periprosthetic osteolysis, the complex underlying network of chemokines is mainly driven by macrophages [34]. The production of polymer wear particles leads to a non-specific macrophage-mediated chronic inflammatory and foreign body reaction [35] in which both local and systemic macrophages are involved. This ultimately leads to dysregulation of bone formation and resorption favouring the latter. Local macrophages are activated by

particles either by cell membrane contact through surface receptors, such as CD11b, CD14, toll-like receptors (TLRs), or through phagocytosis of wear debris. Macrophage activation takes place through different intracellular pathways: myeloid differentiation primary response gene 88 (MyD88) and p38 mitogen-activated protein kinase (MAP kinase) and others which in the end, activate nuclear factors, most importantly NF-κB. Transduction of nuclear factors induces release of pro-inflammatory cytokines (tumour necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, prostaglandin E (PGE)-2, macrophage colony-stimulating factor (M-CSF), receptor activator of NFκB ligand (RANKL), etc.) [36,37]. These factors act in an autocrine and paracrine way [38] to induce a series of biological events such as recruitment of more macrophages and osteoclast precursors, their differentiation [39] and further release of cytokines, some of which have chemotactic properties (chemokines) [40]. These chemokines belong to a large family of active biomolecules [41,42] that are directly dedicated to the migration of monocyte/macrophage lineage cells and other cells. Locally activated macrophages release monocyte chemoattractant protein-1 (MCP-1, also called CCL2) [34]. MCP-1 (human gene 17q11.2) belongs to the γ-chemokine subfamily (C–C chemokines) and is an immediate early stress-responsive factor [43]. MCP-1 is primarily involved in the systemic recruitment of pro-inflammatory cells (monocytes, neutrophils and lymphocytes) [44]. Once released in the bloodstream, MCP-1 binds its receptors (G-protein-coupled receptors) CCR2A and CCR2B (human gene ID 1231), with preference for CCR2B expressed by monocytes and activated natural killer lymphocyte (NK) cells [44–46]. With regards to bone, MCP-1 is also expressed by osteoblasts and osteoclasts. Huang *et al.* [47] have shown *in vitro* that when murine macrophages (RAW cells 264.7) were challenged with ultra-high molecular weight PE (UHMWPE) and PMMA particles, MCP-1 was released at fourfold higher levels than the constitutional level of secretion. Moreover, they showed that the conditioned media-induced chemotaxis of human macrophages and mesenchymal stem cells (MSCs) and that this chemotaxis was blocked with an MCP-1 neutralizing antibody. Similarly, when exposed to PMMA and titanium (Ti) particles, human fibroblasts released increased amounts of MCP-1 [48]. Nakashima *et al.* [27] produced similar findings using human monocytes/macrophages exposed to Ti-alloy and PMMA particles. High levels of MCP-1 as well as macrophage inflammatory protein-1α (MIP-1α, also called CCL3), another chemokine, were found after exposure to these particles. When MCP-1 and MIP-1α neutralizing antibodies were added to the media from cultures exposed to PMMA particles and Ti-alloy, the chemotactic activity of these chemokines was inhibited.

Recent studies have subsequently demonstrated *in vivo* systemic migration of macrophages to wear particles deposited at a remote site. Ren *et al.* [49] using *in vivo* bioluminescent imaging showed systemic trafficking of macrophages to PMMA particles injected into the mouse femoral shaft. Macrophages were also found to migrate to sites with UHMWPE injection: this has been shown using either a single bolus of UHMWPE particles in the femoral shaft [50] or using a continuous infusion of UHMWPE particles through a micro-diffusion pump [22,51,52], a more clinically relevant model. The next step was to link *in vivo* MCP-1 secretion and macrophage recruitment in the presence of particles. In a recent *in vivo* study [23], a single injection of MCP-1 was placed in the femoral shaft of mice and genetically modified reporter macrophages were remotely injected intravenously in the tail vein. The macrophages were genetically modified to express the bioluminescent optical reporter gene firefly luciferase (fluc) so that their migration could be tracked by bioluminescence at different time intervals. Signal intensity and subsequent immunohistochemistry confirmed the systemic recruitment of macrophages to the femur induced by local injection of MCP-1. Furthermore, after injection of the MCP-1 receptor antagonist (acting on the CCR2B receptor) associated with a continuous infusion of UHMWPE particles, the systemic migration of macrophages was dramatically reduced. This indicated that the MCP-1-CCR2 ligand–receptor axis is strongly involved in the particle-induced periprosthetic osteolysis. Bone mineral density demonstrated decreased bone deposition in the group receiving particles but no antagonist; this finding was reversed when either a MCP-1 receptor antagonist was given, or CCR−/− reporter cells were infused in the tail vein. Systemic trafficking of MSCs in the presence of UHMWPE particles was affected by the interruption of the MIP-1α-CCR1 ligand–receptor axis [53]. Taken together, these findings suggest that strategies that interfere with cell recruitment may provide a potential method for modulating the inflammatory reaction to orthopaedic implants and their by-products. Technologies, for example bioactive orthopaedic implant coatings, may have a role to play in increasing prosthesis longevity, by modulating cell trafficking to the bone–implant interface [54].

3. Targeting macrophage polarization as a means to mitigate wear particle-induced osteolysis

3.1. Concept of macrophage polarization

Macrophages are a dynamic and adaptive population of cells that can assume various phenotypes as instructed by signals derived from the local microenvironment [55]. This functional plasticity makes macrophages key regulators of inflammation, immunity and tissue regeneration, and modulation of their activation state is an attractive target for a wide variety of therapeutic interventions [56,57]. Currently, this macrophage plasticity is best understood in the framework known as macrophage polarization [57–59]. The first recognized and best known regulators of macrophage function are $CD4^+$ T helper (Th) lymphocyte subsets. Mirroring the well-known polarization of these Th cells, the macrophage polarization paradigm dictates that under the influence of Th1 and Th2 cell-derived cytokines, macrophages assume two distinct phenotypes known as M1 and M2, or 'classically' and 'alternatively' activated macrophages. Although it has since been recognized that macrophage plasticity represents more a continuum of macrophage polarization states rather than the strict dichotomy suggested by the original macrophage polarization model, this paradigm is still a useful framework for simplifying complex, poorly understood macrophage characteristics [58,60].

Most tissues contain highly specialized macrophage subpopulations. These tissue resident macrophages, or M0 (undifferentiated) macrophages, are responsible for the quiescent removal of apoptotic cells, participate to the regulation of tissue homoeostasis and perform various tissue-specific functions [57].

Full M1 polarization, or classical macrophage activation, can be effectively induced by two signals: the first being the

pro-inflammatory priming cytokine interferon-γ (IFN-γ) secreted by NK or Th1 cells, and the second being the synergistically acting cytokine TNF-α [58]. IFN-γ binds to an IFN-γ receptor, which then signals via the Janus-kinase signal transducer and activator of transcription (JAK-STAT) pathway, leading to activation of the transcription factors STAT1 and IRF5 that are directly responsible for the transcription of M1-related genes [61–63]. In addition, danger signal molecules (danger associated molecular patterns or DAMPs), released from invading pathogens or damaged cells or extracellular matrix, can be recognized by certain TLRs, leading to NF-κB activation and to production of type 1 interferon and other pro-inflammatory factors [64,65]. This activation pathway can act in an auto and paracrine manner, and can partially substitute for IFN-γ in inducing the M1 phenotype [58].

M1 macrophages are effector cells in cell-mediated immunity and in the Th1 cell responses [55–59,66]. In classically activated macrophages, microbicidal effector functions are enhanced by increased production of reactive oxygen and nitrogen species by inducing various components of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and nitric oxide synthase pathways, as well as upregulation of lysosomal enzymes and production of antimicrobial peptides and complement components. As a result, macrophages are able to effectively kill phagocytosed pathogens that might otherwise be resistant to endolysosomal conditions. IFN-γ also sensitizes macrophages to pathogen recognition by upregulating TLRs, inflammasome components, Fc-receptors and some of their signalling machineries. Antigen presentation is enhanced in classically activated macrophages by upregulation of major histocompatibility complex (MHC)- and co-stimulatory molecules as well as by effective antigen processing. M1 activation is further characterized by production of high levels of IL-12 that supports a developing Th1 response; production of other pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-23); inhibition of anti-inflammatory cytokine production; and production of inflammatory chemokines (CCL2, CCL3, CCL4, IL-8, CXCL9, CXCL10 and CXCL11) that recruit neutrophils, monocytes and activated Th1 lymphocytes.

In addition to this classical activation state, macrophages can assume various 'alternative' phenotypes, known as alternatively activated macrophages, or M2 macrophages [67,68]. These alternatively activated macrophages are a heterogeneous group of cells that participate in a wide range of physiological and pathological processes such as Th2-polarized responses, allergy, parasite immunity, tissue healing, homoeostasis and fibrosis.

The first and best-characterized alternative macrophage phenotype occurs when M0 or M1 macrophages are exposed to the Th2 cytokine IL-4 [28,69]. In the context of human macrophages, this macrophage phenotype is referred to as M2a [59,63,67]. IL-4 is produced by eosinophils, mast cells, basophils and activated Th2-lymphocytes. IL-4 binds to an IL-4 receptor which signals through the JAK-STAT6 pathway; many of the M2 genes are upregulated directly by STAT6 [62,63]. The IL-4 receptor also uses other less well-characterized signalling pathways that lead to activation of the peroxisome proliferator-activated receptor-γ and phosphoinositide 3-kinase, that have a direct effect on the transcription of M2-related genes. PPARγ also exerts a direct suppressive effect on the production of inflammatory cytokines mediated by STAT1, activator protein-1 (AP-1) and NF-κB [70,71].

M2a polarization is characterized by suppression of pro-inflammatory cytokine production, a suppressed ability to kill intracellular pathogens via diminished production of microbicidal effector molecules, and suppressed antigen presentation ability, and by production of increased levels of IL-10 instead of IL-12 [57–59,66–68]. Membrane receptors with a scavenger function are upregulated, as are a variety of molecules involved in tissue regeneration, wound healing, granuloma formation and immunity against larger parasites. In mouse M2 macrophages, the metabolism of arginine shifts from production of nitric oxide to production of polyamines used in collagen synthesis, cell proliferation and fibrosis via upregulation of the enzyme arginase 1. In addition, M2 macrophages secrete matrix metalloproteinases (MMPs) as well as a distinct profile of chemokines (CCL17, CCL18, CCL22 and CCL24) specifically recruiting Th2 cells, basophils and eosinophils.

As this original description of alternative macrophage activation, several other distinct macrophage phenotypes have been characterized, including those induced by recognition of immune complexes in combination with a TLR stimulus (M2b) or by macrophages exposure to IL-10, transforming growth factor β or glucocorticoids (M2c) [59,63,67]. Collectively, these last two alternative macrophage phenotypes are distinguished by a lack of extracellular matrix production and high levels of IL-10 production; they are primarily considered to perform immunosuppressive or modulatory functions [58].

The transition from a state of inflammation to tissue healing, regeneration and restoration of function is presumed to be owing to the local dynamic shift in the macrophage phenotype from the inflammatory M1 to various forms of M2 phenotypes [57,58,72]. This switch in macrophage phenotype is achieved either by reprogramming of macrophages already at the location and/or by possibly even selective recruitment of distinct blood monocyte subpopulations, with inherently different ability to differentiate into M1 or M2 macrophages in their tissue environment [73].

3.2. Macrophage activation and polarization in aseptic loosening

As the original descriptions of macrophage and foreign body giant cell infiltrates in the periprosthetic tissues surrounding revised loose TJR implants, it has been recognized that macrophages play a key role in chronic inflammation of orthopaedic implants. Indeed, subsequent studies have confirmed that implant-derived wear particles cause macrophage activation and inflammation *in vitro* and *in vivo* [2,4]. According to the commonly accepted paradigm of aseptic loosening, wear particle-induced and macrophage-mediated inflammation ultimately cause peri-implant osteolysis; particle-activated macrophages secrete chemokines and pro-inflammatory cytokines that lead to further macrophage recruitment, increased osteoclastogenesis and suppression of osteoblast formation and function [16,35,74]. Together these changes create a microenviroment that favours bone resorption over bone formation, thus leading to peri-implant osteolysis and implant loosening.

Although wear particles and macrophage interactions have been investigated extensively, few studies have applied the concept of macrophage polarization to this biomaterial particle-induced macrophage activation. Similarly, few retrieval studies have directly characterized the polarization state of the peri-implant tissue macrophages, although increased production of M1 produced pro-inflammatory and chemotactic

factors, such as inducible nitric oxide synthase (iNOS), TNF-α, IL-1β IL-6, PGE-2, IL-8, CCL2 and CCL3, has been reported [10,12,75–86]. Increased production of characteristic M2 macrophage products such as MMPs, IL-10 and vascular endothelial growth factor have also been described from tissues surrounding loose implants [12,87,88].

Koulouvaris *et al.* [89] performed expression profiling of the peri-implant tissues surrounding loose joint replacement using quantitative real-time polymerase chain reaction (qRT PCR) and found strong increases in the expression of M2 macrophage-related marker genes chitinase 1 (CHIT1) and CCL18 compared with osteoarthritic synovial tissues (controls). By contrast, no difference in the M1-related TNF-α and IL-6 was detected between implants with osteolysis and controls. In addition, both PMMA and Ti particles induced CHIT1 expression in human peripheral blood-derived macrophages. The authors concluded that at least in the end stages of osteolysis, M2-macrophage activation predominates. However, qRT PCR normalizes expression levels to housekeeping genes in the whole tissue used as the sample. Increased expression with respect to the control only reflects higher mRNA production per cell basis, but does not reflect higher numbers of recruited cells producing the corresponding protein.

Using immunohistochemistry and western blotting to detect human leucocyte antigen (HLA)-DR (used as a marker for M1 macrophages) and CD163 (used as a marker for M2 macrophages), Rao *et al.* [28] found an increased ratio of M1/M2 macrophages from peri-implant tissues compared with osteoarthritic synovial tissues. Furthermore, in a series of *in vitro* experiments, Rao *et al*. observed increased iNOS and TNF-α production from PMMA-particle-stimulated mouse bone marrow macrophages using flow cytometry and enzyme-linked immunosorbent assay (ELISA). Results were accentuated if lipopolysaccharide (LPS) was administered together with the PMMA particles. Based on these retrieval and *in vitro* studies, the authors concluded that M1-macrophage phenotype predominates in the peri-implant tissues.

A retrieval study by Jämsen *et al.* (E Jämsen, VP Kouri, J Olkkonen, A Cör, SB Goodman, YT Konttinen, J Pajarinen 2013, unpublished data) profiled the expression of macrophage polarizing cytokines from peri-implant tissues and osteoarthritic control tissues using qRT PCR and immunohistochemistry. Although high expression of several chemokines and osteoclast-related products was found in the peri-implant tissues, no significant production of macrophage-polarizing cytokines IFN-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4, IL-13 or IL-17 could be detected. The authors concluded that other macrophage polarizing and activating signals, such as wear particles *per se* or DAMPs adhering to particles surfaces, rather than classical macrophage-polarizing cytokines, are responsible for the peri-implant tissue macrophage phenotype.

Taken together, the data concerning the macrophage phenotype in the peri-implant tissues is currently controversial. However, considering the large body of studies describing the inflammatory and macrophage-activating nature of biomaterial particles and the inflammatory nature of the peri-implant tissues, it seems likely that M1-like, rather than M2, macrophages predominate. This conclusion is further supported by recent reports describing the role of TLRs in the wear particle recognition and subsequent inflammatory reaction. Maitra *et al.* [90] showed that modified alkane polymers, released from UHMWPE and oxidized by interface tissue cells can directly bind to TLR2 and TLR2/1 dimers and activate pro-inflammatory signalling as indicated by activation of NF-κB. Cobalt ions, commonly released from metal-on-metal implants mimic hypoxia, stimulate reactive oxygen species, upregulate TLR4 and lead to cell death [91]. Furthermore, cobalt ions have been shown to directly bind to and activate TLR4 signalling [92–94]. Exo- or endogenous DAMPs can bind to wear particles surfaces and mediate particle-induced inflammation via TLR signalling [95–98]. As discussed above, TLR signalling, especially via TLR4, is one of the cues that can induce M1-like macrophage phenotype.

3.3. Modulation of macrophage polarization as a means to mitigate wear particle-induced osteolysis

As macrophage polarization has profound impact on expression of TLRs and other pattern-recognition receptors (PRRs), on the macrophages ability to produce pro- and anti-inflammatory cytokines and can impact the macrophage's capacity to support tissue healing, modulation of macrophage polarization and thus its activation state would seem as a viable strategy to mitigate wear particle-induced inflammation. Indeed, evidence is emerging that not only particles *per se* but also macrophage polarization plays an essential role in how macrophages respond to biomaterial particle challenge.

Trindade *et al.* [99] previously reported that IL-4 treatment reduced the production of TNF-α, IL-1β and GM-CSF from PMMA-particle-stimulated human peripheral blood monocyte-derived macrophages in a dose-dependent manner. Im & Han [100] demonstrated similar suppressive effect of IL-4 treatment on production of TNF-α and IL-6 from human peripheral blood monocytes stimulated with retrieved Ti6Al4V alloy wear particles.

More recently, Rao *et al.* [28] investigated the sequential modulation of macrophage phenotype *in vitro* using mouse bone marrow-derived macrophages and found that IL-4 administration even after PMMA particle challenge was sufficient to reduce particle-induced TNF-α production. This effect was more pronounced if particles were delivered together with LPS but reduction in TNF-α production was observed also in the PMMA-only group. Thus, sequential modulation of macrophage phenotype from an M1 phenotype to an M2 phenotype is a possible strategy to reduce particle-induced inflammation; in fact, some aspects of the M2 phenotype, including the production of IL-Ra, Ym1 and Arginase 1 were more evident if macrophages had first been activated into the M1 phenotype with LPS or possibly PMMA particles and then polarized into M2-phenotype with IL-4.

Pajarinen *et al.* [101] compared Ti particle responses of polarized human M0, M1 and M2 macrophages using genome-wide microarray expression profiling, qRT PCR and protein suspension array. In comparison to M0 macrophages, the overall chemotactic and inflammatory responses to Ti particles were greatly enhanced upon challenge of M1 macrophages but effectively suppressed when M2 macrophages were challenged. The authors concluded that the mode in which macrophages responded to particle stimulus is dependent on the polarization status of the macrophages and that induction of M2 polarization might be a means to limit particle-induced macrophage activation.

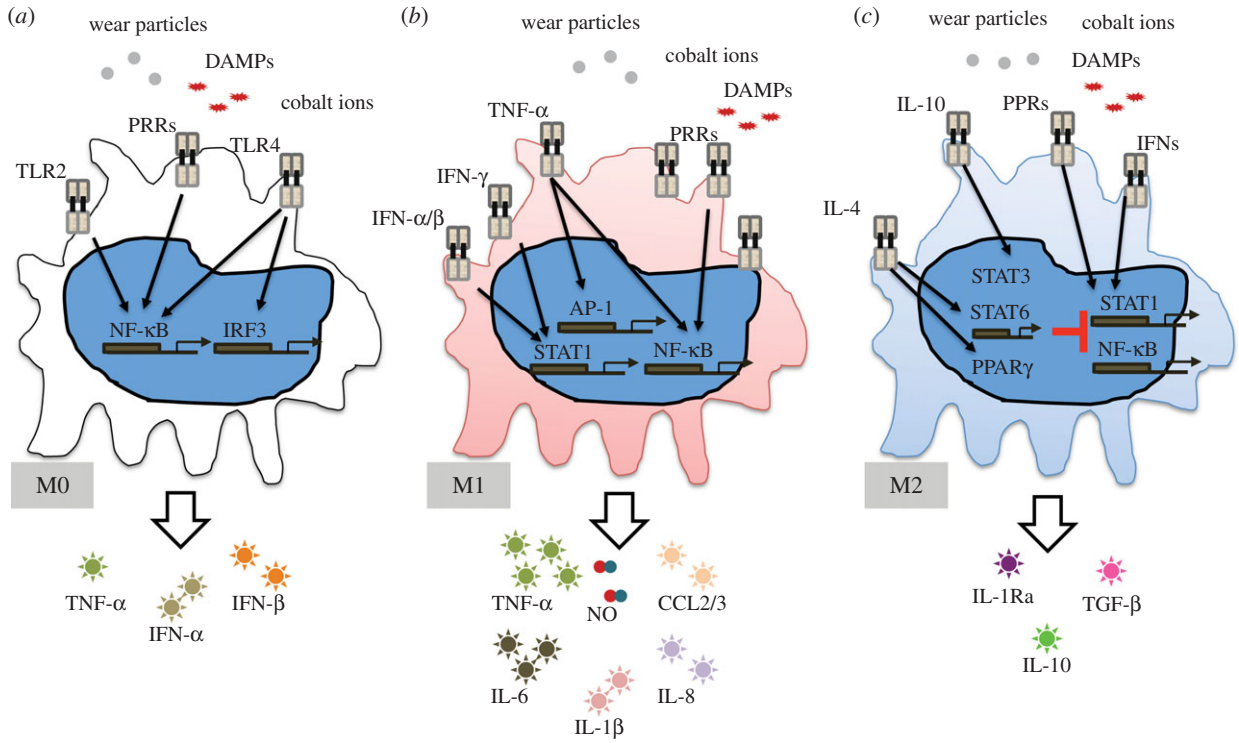

Figure 2. Wear particle-induced macrophage activation in macrophage subsets. (*a*) DAMP-coated wear particles are recognized by TLRs and possibly by other PRRs. Signalling via these receptors leads to NF-κB activation and production of TNF-α and other pro-inflammatory cytokines from M0 macrophages. Activation of TLR4 (for example by LPS or cobalt ions) leads to the production of type 1 interferon via the activation of transcription factor IRF3. (*b*) Type 1 interferon and TNF-α lead to activation of transcription factors STAT1 and AP-1, which are directly responsible for the transcription of M1-related genes. This auto- and paracrine signalling might offer one mechanistic explanation as to why wear particles induce the M1 macrophage phenotype. IFN-γ produced by NK cells or by Th1 cells leads to STAT1 activation which enhances macrophage inflammatory responses by upregulating TLR expression and synergizing with NF-κB; this may explain the exacerbation of wear particle-induced inflammatory response in IFN-γ primed M1 macrophages. (*c*) IL-4 signalling leads to the activation of transcription factor STAT6, which is directly responsible for the transcription of M2 phenotype-related genes. Furthermore, IL-4 signalling leads to activation of PPAR-γ which has direct suppressive effect on NF-κB, AP-1 and STAT1, thus offering a possible explanation as to why the inflammatory wear particle responses in the M2 macrophages are effectively suppressed. M2-polarization is further characterized by production of IL-10, which acting in auto- and paracrine manner, exerts additional suppression of inflammatory transcription factors via activation of STAT3. The mode in which M2 macrophages react to particle stimulus is not yet fully determined.

Antonios *et al.* [102] investigated the dynamics of macrophage polarization and PMMA particle stimulation using mouse bone marrow macrophages. IL-4 treatment reduced PMMA-particle-induced TNF-α production, as previously noted. The effect was most prominent if IL-4 was applied to the cells before, rather than concurrently with the PMMA stimulus; the production of the anti-inflammatory cytokine interleukin-1 receptor antagonist (IL-1Ra) was highest if macrophages had first passed from the M0 to the M1 state before being further polarized into an M2 phenotype.

In addition to these *in vitro* studies, some recent *in vivo* studies have investigated the effect of IL-4 treatment on wear particle-induced osteolysis. Using a mouse calvarial model of particle-induced osteolysis, Rao *et al.* observed that daily IL-4 injections to the subcutaneous bursa overlying the calvaria significantly reduced PE-particle-induced osteolysis as assessed by micro computed tomography (μCT) and histomorphometry [29]. The number of tartrate resistant acid phosphatase (TRAP) positive osteoclasts was reduced to the level of controls in the IL-4-treated group. In addition, prior IL-4 treatment reduced particle-induced TNF-α and RANKL production from calvarial samples cultured *ex vivo*. Using western blot analysis of calvarial proteins using iNOS as M1 marker and Ym1 as M2 marker, increased M1 to M2 ratio was observed in PE particle-treated group while in IL-4 treatment returned this ratio to that of negative controls. Thus, IL-4 treatment reduced particle-induced osteolysis by modulating macrophage activation from M1 towards M2-like macrophage phenotype.

Wang *et al.* [103] observed similar phenomenon in a mouse air pouch model of PE-particle-induced osteolysis; daily IL-4 or IL-13 injections reduced particle-induced bone collagen loss and the area of bone surface covered by TRAP-positive osteoclasts. Corresponding reduction in the production of RANKL and TRAP and increase in osteoprotegerin production was observed in IL-4-treated groups using both RT-PCR and ELISA. Interestingly, these effects were more pronounced if both IL-4 and IL-13 were administered rather than IL-4 or IL-13 alone. However, no analysis of the local macrophage phenotype was performed.

Taken together, current *in vitro* and *in vivo* data would seem to support the hypothesis that macrophage polarization is an essential factor that determines how macrophages respond to biomaterial particle challenge, and that local modulation of the macrophage phenotype appears to be an effective means to limit biomaterial wear particle-induced inflammation and subsequent osteolysis (figure 2).

Prior observations note that not only are the inflammatory wear particle responses suppressed in M2 macrophages, but they are also aggravated in M1 macrophages. This phenomenon was first reported by Trindade *et al.*, who showed that IFN-γ pre-treatment enhances the production of TNF-α and IL-6 from human monocytes stimulated with PMMA particles

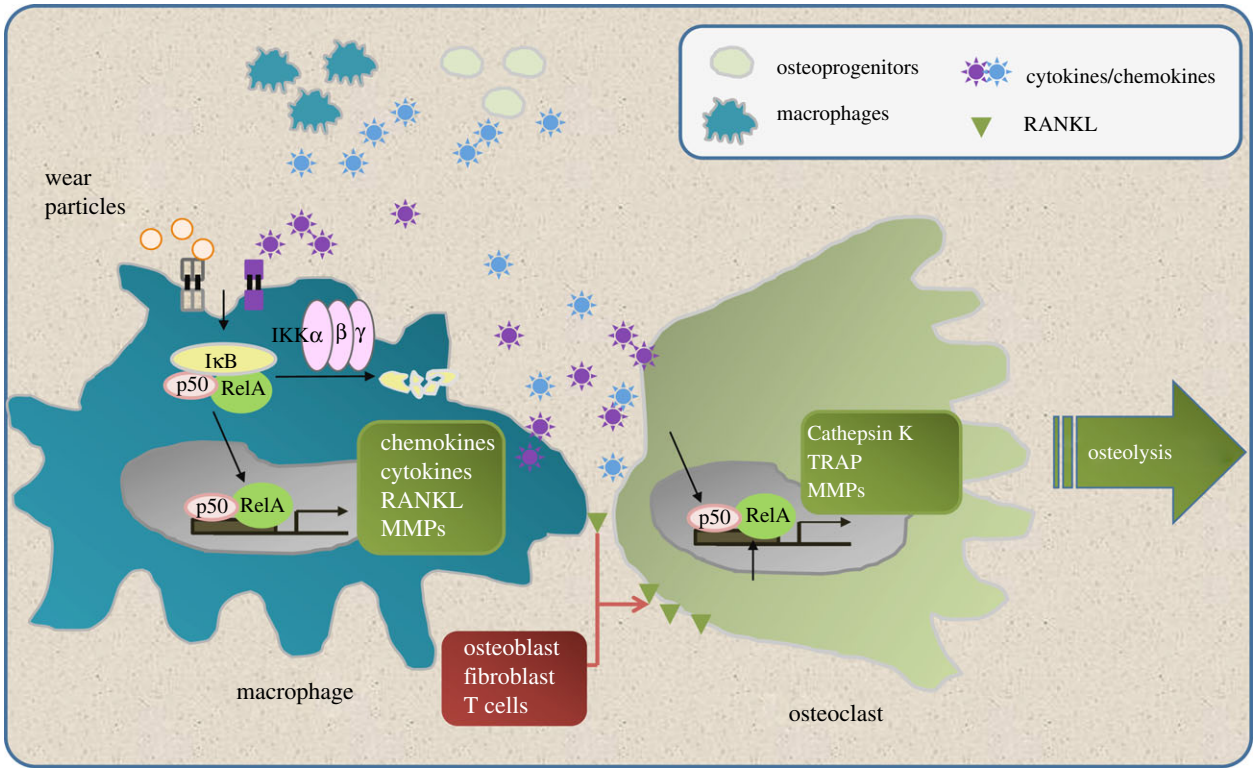

Figure 3. The role of NF-κB in wear particle-induced periprosthetic osteolysis. Inhibition of NF-κB decreases the production and release of pro-inflammatory substances from macrophages, and the differentiation and function of osteoclasts.

and later confirmed by Pajarinen *et al.* showing that the overall inflammatory and chemotactic response to particles was enhanced if macrophages had first been polarized into M1 phenotype [101,104]. The potential significance of these *in vitro* observations remains to be determined; however, they conjure some interesting hypotheses. For example, it is possible that chronic, low-grade inflammation in other anatomic locations caused by atherosclerosis, metabolic syndrome, periodontitis or other conditions might predetermine the systemic M1–M2 balance of macrophages and thus the mode that local macrophages react to wear particles. These other conditions may impact the susceptibility of an individual to develop aseptic osteolysis. Future studies will explore the correlation among individual patient characteristics, macrophage polarization and the reaction to orthopaedic implants and their by-products.

4. Suppression of wear particle-induced periprosthetic osteolysis via direct targeting NF-κB

Despite their essential role in wear particle-induced osteolysis, blocking individual pro-inflammatory cytokines, for example TNF-α, did not mitigate osteolysis in a clinical study [105]. The failure of such therapies may be due to the compensatory role of other wear particle-induced cytokines that can activate or be activated by NF-κB [106,107]. NF-κB signalling can be activated in macrophages and osteoclasts by exposure to wear particles [28], leading to the hypothesis that targeting NF-κB activity could be a potential strategy to mitigate osteolysis (figure 3).

The core transcriptional component of the canonical NF-κB pathway is composed of RelA/p50 heterodimer, which is usually in an inactive state through association with IκB (inhibitor of κB) protein. Once the signal is activated, the IκB protein is phosphorylated by the IKK (IκB kinase) which is then degraded through ubiquitination. The released RelA/p50 dimers then translocate into the nucleus, bind to the response element and modulate the target gene expression. The strategy of direct NF-κB inhibition includes modulation of (i) IKKα/β kinase activity, (ii) IκB protein stability, and (iii) the transactivation of the heterodimer RelA/p50. IKKα/β is one of the most common targets for NF-κB inhibition [108]. Suppression of IKKα/β activity can be achieved by using IKKα/β-specific ATP analogues [109], expression of a dominant negative form [110] or blocking the IKKα/β interaction with IKKγ [111]. A previous study showed that inhibition of IKKα/β kinase activity can block PMMA-particle-induced osteolysis in a murine calvarial model [111]. Targeting IκB via prevention of protein degradation or over-expression of IκB protein is also an efficient strategy to modulate NF-κB activation [110,112].

The NF-κB heterodimer transactivation process includes nuclear translocation, specific DNA binding to the target sequence and enhancement of target gene transcription. Among these, suppression of the DNA binding ability via decoy oligodeoxynucleotide (ODN) is one of the most potent and specific ways to suppress NF-κB transactivation. Decoy ODNs are short synthesized duplex DNAs that mimic the transcription response element and can specifically suppress transcription factor activity via competitive binding with endogenous protein [113]. Although the low bioavailability and short half-life of decoy ODN may limit its clinical application, recent findings suggest that decoy ODNs can be improved by chemical modifications for example replacing the phosphodiester bond with a phosphothioate bond [113]. Application of decoy ODNs has been shown to prevent

NF-κB transactivation of pro-inflammatory cytokine genes in primary cultured macrophage [114]. In addition, NF-κB ODN has also been used in several inflammatory disease animal models, including chronic obstructive pulmonary disease [115], vascular and cardiovascular disease [116–119], liver injury [120], atopic dermatitis [121] and periodontal disease [122].

To optimize the NF-κB inhibition strategy, the delivery route, timing and dosage, and potential toxicity should all be considered. As wear particle-induced osteolysis is generally an anatomically confined disease, local treatment could be an ideal way to prevent the undesired effect on the normal host immune system [123]. On the other hand, blocking NF-κB activation in a confined microenvironment may also suppress the protective effects on osteolysis. For example, the type I collagen in osteoprogenitor cells [124] and the IL-10 secreted by MSCs [125] are both targeted by NF-κB [124,126]. Recent studies have suggested that inhibition of NF-κB can enhance osteogenic differentiation in periodontal ligament stem cells or MSCs via suppression of β-catenin signalling [127–129]. Therefore, the overall beneficial effects among different cell types must be carefully evaluated.

Crosstalk and potential compensatory regulation between the NF-κB pathway and other transcription factors may be critical in order to optimize the therapeutic efficiency of NF-κB ODN. For example, suppression of NF-κB activity may unexpectedly enhance cytokine expression owing to enhanced AP-1 transactivation [130] or block the negative feedback regulator, TNF-α-induced protein 3 [131]. In addition, wear particles can activate NF-κB and nuclear factor interleukin-6 (NF-IL-6) transcriptional activity [31]; both of these transcription factors have essential roles in pro-inflammatory cytokine regulation. Thus, further preclinical studies are necessary to establish the efficacy and safety of local delivery of NF-κB ODN for the treatment of periprosthetic osteolysis.

5. Discussion

The by-products of wear of the bearing surfaces of current TJRs stimulate an inflammatory response that may result in chronic synovitis, periprosthetic osteolysis, implant loosening and pathologic fracture. These processes are initiated in otherwise well-functioning joint replacements. Although many advances have been made in bearing materials, implant technology and design, and surgical technique, revision surgeries due to implant wear are still commonplace. As joint replacements are being extended to younger, more active individuals who will potentially live for many decades, the wear problem will become even more complex in the future. Especially, troublesome is the patient with radiographic wear of their joint replacement and synovitis or early osteolysis, but the implant is not at the point of needing revision surgery. Are there potential interventions to modulate the inflammatory environment to extend the lifetime of the implant in an efficacious and safe manner?

Systemic treatments to date have been shown to be ineffective in humans, and may be associated with adverse effects on other body systems [21,105]. For example, systemic inhibition of specific cytokines such as TNF or IL-1 with biologics may result in blunted reactions to harmful agents such as bacteria and other infective agents, and might even interfere with the eradication of cancer cells. Bisphosphonates inhibit osteoclast-mediated bone degradation associated with particle disease, but may also lead to pathologic femoral fracture, mandibular lesions, impairment of fracture healing and other adverse events [132,133].

Our approach has been to work upstream in the particle-induced inflammatory cascade and use local treatments and interventions, if possible. This has involved interfering with macrophage migration from remote sites to the area of particle generation, changing the local macrophage polarization phenotype from a pro- to an anti-inflammatory one involved in repair and inhibiting the release of numerous pro-inflammatory chemokines, cytokines and other substances by interfering with the transcription factor NF-κB.

Interference with the MCP-1-CCR2 chemokine ligand–receptor systemic is novel and may have a direct clinical application. Surgical placement of a prosthesis evokes an inflammatory reaction initially that may eventually compromise the prosthesis–bone interface. Mitigating this early inflammatory reaction may provide a more robust bone–implant interface initially, which can function as a physical deterrent to particle and inflammatory mediator migration throughout the 'effective joint space' [134]. Potential therapies to accomplish this goal may be delivered as an injected or infused biologic, or a coating on the prosthesis or bone bed during initial prosthesis implantation. Using a layer-by-layer technique or other drug delivery platforms, the biologic may be released according to a desired profile for a time period of seconds to weeks. Further research is necessary to determine the appropriate time period and release profile.

The identification of different macrophage phenotypes is a recent discovery with great potential to modulate the processes involved in inflammation and its resolution [135]. Damage to adjacent normal 'bystander' cells and tissues is an unwanted by-product of the inflammatory cascade. Modulation of the intensity, timing and duration of events associated with acute inflammation, and avoidance of potentially persistent and injurious chronic inflammation could ostensibly alter our conceptual thinking regarding orthopaedic implants. With regards to the prosthesis interface, local delivery of IL-4 or -13 might be a sound therapeutic strategy to convert M1 pro-inflammatory macrophages into an M2 anti-inflammatory pro-tissue healing phenotype [28,29,136]. This could be performed with 'minimally invasive' techniques, for example local injection in the radiology suite; this treatment could potentially be repeated as long as the prosthesis is salvageable.

Once activated, macrophages and other cells in the prosthetic bed demonstrate upregulation of NFκB, the most important transcription factor with over-reaching control over pro-inflammatory factor synthesis and release, and osteoclastogenesis and function. Others have shown that inhibiting NF-κB activation by attenuating the assembly of the IKK complex (using a short peptide termed NEMO-binding domain peptide, that blocks binding of IKK2 and IKK1 to IKKg/NEMO) is effective in inhibiting osteoclastogenesis and PMMA-induced osteolysis in mice [111]. Our novel approach uses an NF-κB decoy ODN to interfere with the functionalization of NF-κB. This ODN can be locally injected, infused or coated onto a surface, and has been shown to be safe and effective *in vitro*. Ongoing *in vivo* studies will help substantiate whether this approach is translatable to the clinical situation.

The continuous production of wear particles is expected, whether the joint is natural or artificial. In general, the body's homoeostatic mechanisms can deal with this foreign material

without the development of overwhelming local chronic inflammation, which may lead to bone loss and implant loosening. The novel strategies outlined above may have a potential role when periprosthetic inflammation is ongoing but the joint replacement is still functional and salvageable.

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