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Do Genetic Susceptibility, Toll-like Receptors, and Pathogen-associated Molecular Patterns Modulate the Effects of Wear?

Edward M. Greenfield PhD

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Abstract

Background Overwhelming evidence supports the concept that wear particles are the primary initiator of aseptic loosening of orthopaedic implants. It is likely, however, that other factors modulate the biologic response to wear particles. This review focuses on three potential other factors: genetic susceptibility, Toll-like receptors (TLRs), and bacterial pathogen-associated molecular patterns (PAMPs).

Where Are We Now? Considerable evidence is emerging that both genetic susceptibility and TLR activation are important factors that modulate the biologic response to wear particles, but it remains controversial whether bacterial PAMPs also do so.

Where Do We Need to Go? Detailed understanding of the roles of these other factors may lead to identification of novel therapeutic targets for patients with aseptic loosening.

How Do We Get There? Highest priority should be given to polymorphism replication studies with large numbers of patients and studies to replicate the reported correlation between bacterial biofilms and the severity of aseptic loosening.

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E. M. Greenfield (✉)

Department of Orthopaedics, Case Western Reserve University,
University Hospitals Case Medical Center, Biomedical Research
Building, Room 331, 2109 Adelbert Road, Cleveland,
OH 44106, USA
e-mail: emg3@cwru.edu

Introduction

Overwhelming evidence supports the concept that wear particles are the primary initiator of aseptic loosening of orthopaedic implants [7]. For example, increased wear of the UHMWPE acetabular cups correlates with an increased rate of osteolysis [44, 55, 111]. However, those studies also showed that other factors modulate the biologic response to wear particles. Also consistent with the importance of other factors is the finding that monocyte production of proinflammatory cytokines in response to polyethylene and titanium wear particles varies greatly between individual donors [31, 46]. The similar finding with different types of particles is not surprising because the different types of particles are thought to induce osteolysis through similar proinflammatory mechanisms [7, 75, 102, 108]. This review focuses on three other factors that may modulate the effects of wear particles: (1) genetic susceptibility; (2) Toll-like receptors (TLRs); and (3) bacterial pathogen-associated molecular patterns (PAMPs).

Where Are We Now?

Genetic Susceptibility

A recent systematic review concluded that multiple genetic polymorphisms are likely to be associated with an increased risk of aseptic loosening [20]. As is the case for most situations where multiple polymorphisms are involved, the contribution of each polymorphism is likely to be relatively small [97, 116]. The polymorphisms that have been reported to associate with aseptic loosening include four genes that encode for proinflammatory cytokines and their antagonists

Table 1. Polymorphisms that have been reported to associate with aseptic loosening

Pathway	Gene	SNP	Odds ratio (CI)	Number of patients		Reference
				Control group	Osteolytic group	
Inflammatory cytokines	TNF α	rs361525	1.8 (1.0–3.2)	267	214	Wilkinson et al., 2003 [112]
Inflammatory cytokines	TNF α	rs361525	6.59 (1.47–29.64)	89	116	Gallo et al., 2009 [27]
Inflammatory cytokines	IL6	rs1800797/rs1800796/ rs1800795	~3	340	270	Gordon et al., 2008 [32]
Inflammatory cytokines	IL6	rs1800795	2.51 (1.27–4.98)	89	116	Gallo et al., 2009 [27]
Inflammatory cytokines	IL6	rs1800797/rs1800796	5.43 (1.73–17.0)	23	22	Kolundzic et al., 2006 [56]
Inflammatory cytokines	IL1RA	rs419598	0.66 (0.48–0.91)	340	270	Gordon et al., 2008 [32]
Inflammatory cytokines	IL-2	rs2069762	0.55 (0.31–0.98)	89	116	Gallo et al., 2009 [27]
RANKL axis	RANK	+575	1.77 (1.20–2.59)	144	85	Malik et al., 2006 [67]
RANKL axis	OPG	rs3102725	3.76 (2.31–6.11)	150	91	Malik et al., 2006 [67]
wnt signaling	sFRP3	rs288326	0.62 (0.38–0.99)	341	268	Gordon et al., 2007 [33]
Lectins	MBL	-550C	2.23 (1.42–3.5)	150	91	Malik et al., 2007 [66]
Lectins	MBL	codon 54	2.17 (1.18–3.98)	150	91	Malik et al., 2007 [66]
Matrix degradation	MMP1	rs58554	3.27 (2.21–4.83)	148	87	Malik et al., 2007 [68]
Matrix degradation	MMP1	-1607	~4	31	27	Godoy-Santos et al., 2009 [30]
Purinergic receptors	P2RX7	rs28360457 + rs1653624 + rs35933842	4.96 (0.64–38.6)	48	157	Mrazek et al., 2010 [72]
Growth factors	TGF β 1		8.23 (1.48–46.8)	23	22	Kolundzic et al., 2006 [56]
Guanine nucleotide binding proteins	GNAS	T393C	Males: 0.088 (0.02–0.50) Females: 2.2 (0.86–5.43)	NA	37 males + 20 females	Bachman et al., 2008 [4]

SNP = single nucleotide polymorphism; CI = confidence interval; RANKL = receptor activator of NF- κ B ligand; wnt = wingless-type MMTV integration site family; TNF = tumor necrosis factor; IL = interleukin; OPG = osteoprotegerin; MMP = matrix metalloproteinase; GNAS = guanine nucleotide binding protein α -subunit; NA = not applicable.

(Table 1). Importantly, two of these genes (tumor necrosis factor [TNF] α and interleukin [IL] 6) are the only examples in which multiple groups of investigators have reported that the same polymorphism associates with aseptic loosening [27, 32, 56, 112]. Moreover, the polymorphisms in those genes also associate with many other inflammatory conditions [26, 86]. Those polymorphisms are located in the promoter regions of their respective genes and can regulate their transcription [26, 53, 92, 99, 105].

Polymorphisms in two genes that encode for members of the receptor that activates NF- κ B (RANK) ligand (RANKL) axis are also shown (Table 1). Those associations with aseptic loosening, in combination with those in genes that encode for proinflammatory cytokines, confirm the well-accepted concept [35, 38, 75, 108] that aseptic loosening is primarily driven by the following stepwise process: (1) wear particles induce production of

proinflammatory cytokines; (2) the proinflammatory cytokines stimulate production of RANKL; (3) RANKL increases osteoclast differentiation; and (4) the increased number of osteoclasts causes local osteolysis.

In addition, polymorphisms in six genes that encode for miscellaneous proteins are shown (Table 1). Those proteins may therefore also contribute to aseptic loosening. For example, a polymorphism in secreted frizzled-related protein 3 (sFRP3) associates with aseptic loosening [33]. sFRP3 is an inhibitor of signaling by the wingless-type MMTV integration site family (wnt) pathway. Because wnt signaling potently regulates the balance between bone formation and bone resorption [50], one possibility is that reduced wnt signaling during aseptic loosening further skews that balance against bone formation. Consistent with that possibility, antibodies that neutralize sclerostin, an inhibitor of wnt signaling, block the negative effect of

polyethylene particles on implant fixation in rats by increasing bone formation and decreasing bone resorption [60]. Also consistent with that possibility, the number of osteoblasts is reduced on bone surfaces surrounding loose implants [49]; patients with high rates of bone formation have lower rates of aseptic loosening [55]; wear particles can reduce osteogenesis in vitro and in animal models [17, 108]; and osteoblasts rapidly repair osteolysis induced by wear particles in young mice [48]. Another possibility is that altered wnt signaling results in skeletal anatomy that predisposes the patient to loosening. Consistent with that possibility, the polymorphism in sFRP3 that associates with aseptic loosening [33] also associates with shape of the femur [5] and specific femoral shapes predispose patients to loosening [55].

More credence should be given to the studies that include a larger number of patients (Table 1). However, sample sizes in the thousands are usually needed to detect associations with moderate effect sizes [97, 116]. Thus, even the largest studies on aseptic loosening should therefore be considered preliminary (Table 1). More credence should also be given to the studies that report narrow confidence intervals (CIs) around the odds ratios (Table 1). However, it is not always clear how those CIs were determined or whether the odds ratios were adjusted for potential confounders. In regard to these issues, the largest studies are those from Wilkinson and colleagues [32, 33, 112] and their odds ratios were adjusted for age, sex, time since primary surgery, and amount of polyethylene wear. An additional important confounder that must be considered in genetic association studies is the degree of genetic heterogeneity within the patient populations [97, 116]. In this regard, all but one [30] of the genetic association studies focus exclusively on European whites (Table 1).

In summary, genetic polymorphisms are likely to be important modulators of the biologic response to wear particles, but which genes are involved remains preliminary.

Toll-like Receptors

The TLRs are a family of pathogen recognition receptors that are responsible for activating the immune system in many situations [51]. The TLR family can conceptually be divided between those that occur primarily at the cell surface (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11) and those that are primarily located on endosomes (TLR3, TLR7, TLR8, and TLR9) [51]. Despite this complexity, most of the studies on the roles of TLRs in aseptic loosening have focused on TLR2 and TLR4. The structure of those TLRs, their coreceptors, and adaptor proteins are shown (Fig. 1). Multiple lines of evidence indicate that activation of TLRs is likely to be another factor that regulates the

Fig. 1 TLR2, TLR4, their coreceptors, and adaptor proteins are shown. TLR2 heterodimerizes with either TLR1 or TLR6 as shown on the left side of the diagram. TLR4 homodimerizes as shown on the right side of the diagram. TLRs are depicted by black objects, their coreceptors are depicted by cross-hatched objects, and their adaptor proteins are depicted by white objects. See text for details. MD2 = myeloid differentiation protein-2; CD14 = monocyte differentiation antigen CD14; TRAM = Toll-like receptor adaptor molecule; TRIF = Toll-interleukin 1 receptor domain-containing adapter protein inducing interferon-beta.

biologic reaction to wear particles. Consistent with this possibility, macrophages in periprosthetic tissues, like macrophages in other tissues, constitutively express a diverse array of TLRs [57, 80, 103]. It is intriguing that oxidized alkane polymers derived from UHMWPE particles can accumulate in periprosthetic tissue and can activate TLR1/TLR2 heterodimers but cannot activate TLR3 or TLR4 [64, 65]. Moreover, it has recently been reported that human TLR4 can be activated by cobalt or nickel ions [88, 110], both of which can be released by corrosion of cobalt-chromium implants [39]. Functional studies have shown that deletion of TLR2 and/or TLR4 in murine systems substantially reduces the in vitro and in vivo responses to titanium [8, 37], UHMWPE [40], cobalt-chromium [85], and hydroxyapatite [34] particles. Moreover, similar results have also been obtained for deletion of the myeloid differentiation primary response gene (MyD88) with both PMMA and cobalt-chromium particles [82, 85]. MyD88 is an adaptor protein involved in signaling by most TLRs and members of the IL-1 receptor family [51, 79].

In summary, TLRs are likely to be important modulators of the biologic response to wear particles, but which specific TLRs are involved remains preliminary. This conclusion however does not exclude the possibility that macrophages likely can also respond to wear particles independently of TLRs.

Bacterial Pathogen-associated Molecular Patterns

PAMPs, which are also known as microbial-associated molecular patterns and were previously known as bacterial

endotoxins, are the best characterized activators of the TLRs discussed in the previous section. For example, lipopolysaccharide (LPS) from Gram-negative bacteria activates TLR4 and lipotechoic acid from Gram-positive bacteria activates TLR2. Considerable evidence exists that adherence of bacterial PAMPs to wear particles can increase the biologic activity of particles consisting of all relevant orthopaedic materials [36, 43, 59, 62], including polyethylene [1, 15, 19, 45, 100, 114]. Importantly, the methods used to remove the PAMPs do not detectably change the particle size, shape, or chemical composition of their surfaces [22, 87]. One interpretation of these results is that PAMPs from bacteria that are present at subclinical levels contribute to aseptic loosening of orthopaedic implants, which by definition occurs in the absence of clinically detectable levels of bacteria. Consistent with that interpretation, antibiotics reduce the rate of aseptic loosening [10, 23] and periprosthetic inflammation [89] in patients as well as particle-induced osteolysis in mice [91]. However, those results do not conclusively support the PAMP hypothesis because antibiotics can have antiinflammatory effects that are independent of their bactericidal effects [58, 61, 90, 94].

The PAMP hypothesis depends on the presence of these molecules, at least episodically, during aseptic loosening. In support of this, LPS has been detected in periprosthetic tissue from patients with inflammatory arthritis and aseptic loosening [73]. Moreover, peptidoglycan, a PAMP produced by both Gram-negative and Gram-positive bacteria, exists in synovial tissue from patients with osteoarthritis and rheumatoid arthritis [16, 96] and, therefore, likely exists in periprosthetic tissue of patients with aseptic loosening. One potential source of PAMPs during aseptic loosening is the bacteria present in the biofilms found on many loose implants despite the absence of clinical signs of infection [21, 47, 54, 74, 76, 107, 109]. Patients with inflammatory arthritis, especially those receiving anti-TNF- α therapies, may be especially at risk for formation of these bacterial biofilms [95]. Importantly, it has recently been reported that presence of the biofilms correlates with the severity of aseptic loosening [98]. Thus, biofilms were detected on 76% of the implants with extensive osteolysis compared with 30% of the implants with less osteolysis. Although that study involved a relatively small number of patients from a single medical center, it suggests that PAMPs from the biofilm contribute to loosening in the absence of clinical signs of infection. Another potential source of PAMPs during aseptic loosening is bacteria present in other locations in the body such as the oral cavity and the gastrointestinal tract. For example, dental bacteria can cause episodic bacteremia and release LPS into the circulation and may also seed biofilm formation on

orthopaedic implants [29, 77, 78]. Moreover, there is extensive evidence that PAMPs can translocate from the gastrointestinal tract to distant organs, including the bone marrow, and systemically prime the innate immune system in the absence of infection [14, 18, 25, 28, 42, 63, 70, 71, 84, 101, 115]. This translocation of PAMPs from the gut can be increased by minor surgical procedures, such as colonoscopy, a high-fat diet, or even a single high-fat meal [3, 14, 52, 71, 84]. Also consistent with the possibility that circulating PAMPs could traffic to the periprosthetic tissue and induce inflammation locally are the findings that total joint arthroplasty increases circulating LPS levels [113] and that LPS from the circulation accumulates around “endotoxin-free” particles after implantation in rodents [104, 114]. The local effects of the PAMPs can also include increased corrosion of titanium surfaces, which in turn can increase PAMP binding to the surface [6]. Alternatively, gut-derived PAMPs could induce chronic low-level inflammation systemically [14, 18, 25, 28, 42, 52, 63, 70, 71, 84, 101, 115], as appears to occur in the synovium during rheumatoid arthritis [12, 106]. In this regard, it has recently been proposed that systemic low-level inflammation may contribute to aseptic loosening [81]. A final potential source of PAMPs during aseptic loosening is contamination of the surface of implants during the manufacturing process [2, 11, 41, 59, 93]. Contaminating PAMPs can also impair the initial osseointegration of the implants [2, 11, 41, 59], which can in turn predispose patients to aseptic loosening [83].

The major source of controversy regarding the PAMP hypothesis is that TLRs can be activated by molecules other than PAMPs. As previously mentioned, oxidized alkane polymers from UHMWPE particles found in periprosthetic tissue can activate TLR1/TLR2 [64, 65]. Moreover, there is extensive evidence that endogenous alarmins (also known as danger-associated molecular patterns) can activate TLRs [9, 69]. A number of investigators have therefore concluded that TLR activation during aseptic loosening is primarily the result of alarmins rather than PAMPs [13, 40, 57, 75, 82]. For example, one of those papers concluded that UHMWPE particles induce production by monocytes of an alarmin known as hsp70 that, in turn, activates TLR4 [40]. In evaluating the alarmin hypothesis (Fig. 2A), it is important to consider that many of the reported effects of the alarmins have now been shown to be the result of contamination with PAMPs and a recent review [24] therefore concluded that “many of these molecules may be more accurately described as PAMP-binding molecules or PAMP-sensitizing molecules, rather than genuine ligands of TLR2 or TLR4.” This perspective would suggest that alarmins and PAMPs likely work together to activate TLRs during aseptic loosening.

Fig. 2A–B Two possible mechanisms of TLR activation during aseptic loosening are shown: **(A)** alarmins are sufficient to activate TLRs, but PAMPs are not required but can contribute and **(B)** PAMPs are required to activate TLRs, but alarmins are not required but can

contribute. See text for details. Solid arrows indicate primary mechanisms of TLR activation. Dotted arrows indicate additional mechanisms that may contribute to TLR activation. adh = adherent.

Consistent with that view, responses to titanium wear particles that involve TLR2 or TLR4 have been shown to require adherence of cognate PAMPs to the wear particles [37]. Those results are strong, albeit indirect, evidence that the alarmins are not sufficient to activate those TLRs in the absence of PAMPs in the cell culture and murine models that were studied (Fig. 2B). It should be emphasized that alarmins may nonetheless contribute to aseptic loosening either by acting together with PAMPs to activate the TLRs or by other mechanisms that are independent of TLRs (Fig. 2B). For example, alarmins may contribute to inflammasome activation by wear particles [13, 57, 75]. However, the relative importance of alarmins and PAMPs remains unknown in patients with aseptic loosening. In summary, the continuum model (Fig. 3) proposes that TLR activation during aseptic loosening is attributable primarily to alarmins in some patients, to PAMPs in some patients, and both alarmins and PAMPs contribute in other patients.

Where Do We Need to Go?

A full understanding of the effects of genetic polymorphisms on aseptic loosening may ultimately lead to identification of susceptible patients. However, it is more likely, at least for the foreseeable future, that further study of polymorphisms will demonstrate the involvement in aseptic loosening of specific

proteins and the biologic processes that they mediate. Such proteins and pathways may therefore represent novel therapeutic targets for patients with aseptic loosening.

Future studies are needed to determine the percentage of patients that are in each group described in the continuum model (Fig. 3) and whether the reported effects of antibiotics on aseptic loosening [10, 23, 89, 91] are the result of bactericidal effects or the antiinflammatory effects of antibiotics that are independent of bacteria [58, 61, 90, 94]. Further in vivo murine studies are also needed to determine (1) whether PAMPs are required for TLR activation; (2) the roles of pathogen recognition receptors other than TLR2 and TLR4; and (3) the mechanisms responsible for particle-induced osteolysis in the absence of PAMPs.

How Do We Get There?

Highest priority should be given to polymorphism replication studies with larger numbers of patients. Genome-wide association studies would be especially useful for identifying novel targets because they are unbiased assessment of polymorphisms throughout the genome. In contrast, all of the studies reported to date have focused on polymorphisms in candidate genes. Followup studies in populations with different genetic backgrounds will then be needed to assess whether the results with European whites can be generalized.

Continuum Model of TLR activation during Aseptic Loosening

Fig. 3 The continuum model of TLR activation during aseptic loosening is shown. White regions indicate the proportion of patients in which alarmins are primarily responsible for TLR activation. Black regions indicate proportion of patients in which PAMPs are primarily responsible for TLR activation. Gray regions indicate proportion of patients in which both alarmins and PAMPs contribute to TLR activation. It is unknown whether PAMPs are important in many patients (top panel), some patients (second panel), few patients (third panel), or no patients (bottom panel).

Highest priority should also be given to studies to replicate the reported correlation between bacterial biofilms and the severity of aseptic loosening [98]. Side-by-side testing of antibiotics with the available structurally related molecules that lack the bactericidal effects but retain the antiinflammatory effects [58] are needed to resolve whether the results with antibiotics [10, 23, 89, 91] provide strong support of the PAMP hypothesis.

Discussion

Although there is overwhelming evidence that wear particles are the primary initiators of aseptic loosening, it is likely that many other factors modulate the biologic response to these particles. Strong evidence is emerging that both genetic susceptibility and TLR activation are two such factors, but it remains controversial whether bacterial PAMPs also modulate the biologic response to wear particles. Detailed understanding of the roles of these other factors may lead to identification of novel therapeutic targets for patients with aseptic loosening.

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