Innate Immune Response and Implant Loosening: Interferon Gamma is Inversely Associated With Early Migration of Total Knee Prostheses

Monique A. E. Schoeman,1 Bart G. Pijls,1 Angela E. Oostlander,1 Johan C. Keurentjes,1 Edward R. Valstar,1,2 Rob G. H. H. Nelissen,1 Ingrid Meulenbelt3

1Department of Orthopaedics, Leiden University Medical Center, Leiden, The Netherlands; 2Department of Biomechanical Engineering, Faculty of Mechanical, Maritime, and Materials Engineering, Delft University of Technology, Delft, The Netherlands; 3Department of Molecular Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

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ABSTRACT: To allow prediction of the risk of loosening prior to surgery, we investigated the relationship between innate immune cytokine response via TLR2 stimulation and early migration of six different knee prostheses using RSA (radiostereometry). This study included 114 patients of a prospective RSA-cohort who received a total knee arthroplasty. Whole blood cytokine responses were obtained by ex vivo stimulation with triptanolyl-S-glyceryllyssteine (Pam3Cys-SK4) for assessment of the TLR2 immune response. Early migration was calculated using the maximum total point motion (MTPM) 1 year post surgery. Principal component analysis (PCA) was applied to the cytokine data to reduce the correlated data of individual cytokines and identified two components. Subsequently, linear mixed model analyses were applied with adjustments for gender, age, BMI, time-to-blood sampling, and prosthesis type. Component 1, consisting of IFNγ, IL-12p40, IL-10, IL-1β, TNFα, and IL-6, showed a significant inverse association (β = −0.128; p = 0.041) with MTPM. Further analysis showed that IFNγ (β = −0.161, p = 0.008) had the highest contribution to this association and is particularly found in patients receiving another prosthesis than Nexgen (β = −0.239; p < 0.001). In conclusion, patients with high levels of IFNγ upon stimulation of TLR2 are at lower risk of early migration of their knee prosthesis. © 2015 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 34:121–126, 2016.

Keywords: aseptic loosening; migration; IFNγ; TLR2

Aseptic loosening is the most common cause for failure in total joint arthroplasty at long-term follow-up.1,2 Mechanical as well as biological factors play an important role in the loosening process. Inadequate initial fixation and repeated strains and stresses during normal gait cycles affect the bone-implant interface.3,4 On the other hand, loss of fixation can be caused by particle-induced osteolysis around the implant. Particular wear debris, continuously generated by articulation motion at the bearing surfaces, is thought to trigger an aseptic inflammatory response and cause an increase in osteoclast activity and subsequent peri-prosthetic bone resorption.5 In vitro studies have shown that wear particles stimulate macrophages to produce cytokines including tumor necrosis factor α (TNFα), interleukin (IL)-1β, and IL-6.6–9 Consistent with these in vitro findings, several clinical studies have demonstrated increased production of these cytokines in peri-prosthetic tissues and fluids of loosened prostheses.9–11

The mechanism of the initial cellular interaction with wear particles and the subsequent production of inflammatory mediators is still largely unknown. Recent studies suggest a critical role of Toll-like receptors (TLRs) in this process.12–14 TLRs belong to a class of receptors that enable the innate immune system to recognize foreign material and to mediate inflammatory responses.15 It is known that different TLRs detect different pathogen associated molecular patterns (PAMPs). TLR2 mainly detects lipoproteins whereas TLR4 binds lipopolysaccharides (LPS) that are part of the membrane of gram-negative bacteria.15 Both TLRs have been shown to be present in peri-prosthetic tissue of patients with aseptic loosening.16 Furthermore, TLR2 has been shown to recognize wear particles and to mediate the subsequent inflammatory reaction via the TLR2 specific, MyD88 dependent signaling pathway.12,13,17 However, a large variation in the response to wear particles both in magnitude as well as the inflammatory cytokine profile between individuals has been observed.18 Hence, this raises the question whether diversity in clinical susceptibility to wear particles can be explained by variations in cytokine release due to differences in innate immune responses.3 Genetic studies have already shown that associations between aseptic loosening and polymorphisms in cytokine genes exist.19,20

Early detection of loosening, even before symptoms occur, is possible by measuring sub millimeter migration of the prosthesis relative to the host bone.21–23 Radiostereometric analysis (RSA) allows in vivo three-dimensional measurement of prosthetic migration with a high level of accuracy.22–24 Studies have shown that increased early migration (1 or 2 years postsurgery) is associated with increased risk of aseptic loosening and subsequent revision at the long term.23,25 However, RSA measurements require the insertion of 1 mm tantalum in the patient’s bone. Therefore, RSA can only be used to early assess loosening in patients that have been included in clinical RSA studies. Since we hypothesize that differences in innate immunity are related to the susceptibility of aseptic loosening, markers of the innate immunity might be suitable as predictors for loosening
even before the patient receives joint replacement surgery.

Therefore, the aim of this study was to investigate whether there is an association between innate immunity, as reflected by the capacity to produce cytokine responses upon stimulation with the TLR2 agonist Pam3Cys-SK4, and early prosthesis migration, as measured by RSA in a cohort of patients with a total knee arthroplasty (TKA).

MATERIALS AND METHODS

Study design (Level of Evidence): This is a cross-sectional study (Level III).

Patients
Our cohort consisted of 137 patients who received a total knee arthroplasty (TKA) and were included previously in one of our prospective clinical RSA studies. Between February 2010 and June 2011, all patients in our cohort visited the Leiden University Medical Center for routine RSA measurements and collection of blood samples. Twenty-three patients were excluded from this study for various reasons, see Figure 1. In total, 34 patients received a bilateral TKA. However, from eleven of these patients only one knee was included in the study as the other prosthesis was less than one year in situ. As a result, in the current study we have included 114 patients with 137 knee prostheses. Within this cohort, patients received different designs of knee prostheses, including Nexgen (Zimmer, Inc.), Triathlon (Stryker, Inc.), Rocc (Biomet, Inc.), Interax, Interax ISA, Interax PS (Howmedica Osteonics Corp).22,26–28 The study was approved by the Medical Ethical Committee of the Leiden University Medical Center (P09.228), and informed consent was obtained from all patients.

RSA Analysis
RSA radiographs were made in a uniplanar setup using a highly accurate carbon calibration box (Carbon box, Leiden, The Netherlands) positioned underneath the examination table. The first RSA examination was made before weight-bearing on the second postoperative day and served as the reference for all further examinations. All evaluations are related to the relative position of the prosthesis to the bone at that time. The RSA data was analyzed using commercially available software (Model-based RSA, version 3.34, RSAcore, LUMC, Leiden, The Netherlands) and enables determination of the relative 3D position of the markers of the prosthesis in relation to the bone markers. In situations where less than three markers could be detected, the Marker Configuration Model RSA technique was used.29 The parameter indicating the largest threedimensional migration of any point on the prosthesis surface is called Maximal Total Point Motion (MTPM).24 The reason for using MTPM as measure for migration, other than translation and rotation of the prosthesis, is that motion implies a biological effect and this effect is liable to be greatest at the point of maximum motion. To measure early migration of the prosthesis, MTPM of the tibial component was assessed 1 year postoperatively.

Cytokine Measurement
The innate immune response of patients was assessed by measuring the cytokine production capacity of whole blood samples upon ex vivo stimulation as described elsewhere.30 The cytokine production capacity was assessed by ex vivo stimulation of 2 ml of whole blood with Pam3Cys-SK4, which stimulates the TLR2 response. Blood was collected in heparinized tubes and samples were diluted twofold with RPMI-1640 (Sigma, St. Louis, MO). Samples were incubated for 24 h with 25 μg/ml N-Palmitoyl- S-[2,3-bis[palmitoyloxy]- (2RS)-propyl]-[R]-cysteinyl-(S)-seryl-(S)-lysyl-(S)-lysyl-(S)-lysine (EMC Microcollections, Tübingen, Germany) at 37°C and 5% CO₂. After centrifugation, the supernatants were stored at −80°C until assayed for IL-4, IL-13, IFNγ, GM-CSF, IL-6, TNFα, IL-12p40, IL-1ra, TGFβ, IL-10, and IL-1β using standard ELISA techniques according to manufacturers’ guidelines (Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands). Data on IL-4, IL-13, and GM-CSF production upon stimulation was not available, since in most samples, levels of these cytokines were below the detection limit (4 pg/μl for IL-4 and IL-13; 30 pg/μl for GM-CSF).

Statistical Analysis
Due to the non-normal distribution of MTPM values and cytokine levels, a log transformation was applied to these variables in all statistical models applied. A principal component analysis (PCA) was performed to reduce the multitude of correlated cytokine variables by creating “summary” variables, so called principal components.31 Data of IFNγ, IL-6, TNFα, IL-12p40, IL-1ra, TGFβ, IL-10, and IL-1β were entered in the PCA. Percentage of variance explained by the components and Eigen values >1 were used to determine the number of principal components. For each variable in the extracted component(s), a “factor loading” was calculated which can be interpreted as correlation measure between the observed variable and the component. For analysis, only factor loading scores of >0.4 (significant) qualified for loading of a variable on a component.32
In order to assess the relationship between early migration and the innate immune response, a multivariate generalized estimating equations (GEE) model was fitted with MTPM as dependent variable and the extracted principal components as independent variables. In all analyses, gender, age, body mass index (BMI), time-to-blood sampling, and prosthesis type were added as covariables. Since every knee has its own MTPM value, patients with bilateral knee replacements have two MTPM values but only one data-set of cytokine responses. To model the intrapatient MTPM correlation in patients with bilateral knee replacement, family identity numbers were included as random effect variables. Results of the GEE model analyses are expressed as estimates ($\beta$) that represent the association between MTPM and extracted principal components of cytokine levels. Statistical analyses were performed using SPSS version 20 (SPSS, Chicago, IL, USA). 

$p$-values $<0.05$ were regarded as statistically significant.

RESULTS

Baseline patient characteristics of our study are presented in Table 1. Maximal total point motion (MTPM) values of 137 knees in 114 patients were available. Median MTPM was 0.66 mm and had a range of 0.15–8.19 mm. Cytokine levels were measured from whole blood samples collected one to 17 years postoperative. Table 2 shows the cytokine levels of whole blood samples stimulated with TLR2 agonist Pam3Cys-SK4. As cytokine responses are likely to act simultaneously in the innate immune response, high correlation between secreted cytokines (see Supplementary Table S1) was observed. To reduce the correlated data of the cytokines principal component analysis (PCA) was performed on all cytokines for which a quantitative measure was available. As shown in Table 2, two components were extracted. The first component is determined by a combination of IFN$\gamma$, IL-12p40, IL-10, IL-1$\beta$, TNF$\alpha$, and IL-6, and explains 53.0% of the total variation. The second component, explaining 15.4% of the total variation, is determined by a combination of TGF$\beta$, IL-1$\alpha$, and IL-10.

Next, we investigated the relationship between MTPM and the principal components extracted. We observed a significant and independent inverse association between MTPM and the first component ($\beta=-0.128; p=0.041$), however, the association with the second component ($\beta=0.007; p=0.911$) was not significant. Respective effect sizes of covariables age, gender, BMI, time-to-blood sampling, and prosthesis type are shown in Supplementary Table S2; model 1.

To explore whether the cytokine(s) of component 1 contributed equally to the association with MTPM, we performed a multivariate analysis with MTPM as dependent variable and the cytokines from component 1 as

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Pam3Cys-SK4 (pg/ml)$^a$</th>
<th>Component$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN$\gamma$</td>
<td>33 (40)</td>
<td>1 0.69</td>
</tr>
<tr>
<td>TGF$\beta$1</td>
<td>2737 (1432)</td>
<td>1 0.79</td>
</tr>
<tr>
<td>IL-12p40</td>
<td>279 (545)</td>
<td>1 0.82</td>
</tr>
<tr>
<td>IL-1$\alpha$</td>
<td>16242 (10661)</td>
<td>1 0.71</td>
</tr>
<tr>
<td>IL-10</td>
<td>276 (432)</td>
<td>1 0.81</td>
</tr>
<tr>
<td>IL-1$\beta$</td>
<td>118 (180)</td>
<td>1 0.64</td>
</tr>
<tr>
<td>TNF$\alpha$</td>
<td>148 (446)</td>
<td>2 0.86</td>
</tr>
<tr>
<td>IL-6</td>
<td>6189 (12770)</td>
<td>2 0.84</td>
</tr>
</tbody>
</table>

$^a$Data are expressed as median (IQR).$^b$Components with “Eigen values” >1 are extracted after Varimax rotation with Kaiser Normalization, significant coefficients with values >0.4 are displayed.

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**Table 1.** Study Characteristics

| Data are expressed as N or mean ± SD. Data of MTPM are expressed as median (range) in mm. $^a$Mean number of years between surgery and blood sample collection.

**Table 2.** Principal Component Analysis of Cytokines

<table>
<thead>
<tr>
<th>Component</th>
<th>Variance explained (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53.0</td>
</tr>
<tr>
<td>2</td>
<td>15.4</td>
</tr>
</tbody>
</table>

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INNATE IMMUNE RESPONSE AND IMPLANT LOOSENING
separate covariates. We found a significant inverse association with MTPM only for IFNγ (β = −0.161; p = 0.008), which was independent of the other cytokine levels and age, gender, BMI, time-to-blood sampling, and prosthesis type. Respective effect sizes of covariates other cytokines, age, gender, BMI, time-to-blood sampling, and the prostheses types are shown in Supplementary Table S2; models 2 and 3.

Finally, it was assessed whether the association of IFNγ with MTPM showed interaction with prosthesis type. A significant interaction between IFNγ and prosthesis type was observed only for Nexgen (β = 0.337; p = 0.019), independent of the covariates age, gender, time-to-blood sampling, and BMI (Supplementary Table S2, model 4), indicating that the relationship between IFNγ and MTPM among patients with a Nexgen prosthesis is different when compared to patients with other prostheses types. Stratified analyses, by “Nexgen” and “other” prostheses types, subsequently showed in the “other” strata an inverse association between IFNγ and MTPM (β = −0.239; p < 0.001), whereas no association was observed in the “Nexgen” strata (β = −0.006; p = 0.938), independent of the covariates age, gender, time-to-blood sampling, and BMI. Exclusion of uncemented prosthesis, all belonging to the “other” strata, did not change the observed inverse association between IFNγ and MTPM.

**DISCUSSION**

In the current study, the association between three-dimensional migration of knee prostheses as measured with RSA and the innate immune responses via specific stimulation of TLR2 was evaluated in a cohort of total knee arthroplasty (TKA) patients. We observed a beta of −0.128, representing an inverse association of the cytokines IFNγ, IL-12p40, IL-10, IL-1β, TNFα, and IL-6, clustered in component 1 of the PCA, with respect to MTPM. Subsequent multivariate analysis showed that IFNγ independently confers this inverse effect on MTPM particularly among patients who received a prosthesis type other than Nexgen (β = −0.239; p < 0.001). It should, however, be noted that the Nexgen prosthesis occurred most frequent in our cohort and had, therefore, the highest power in the interaction analyses. Since studies have shown that increased early migration expressed by increased MTPM is associated with increased loosening and revision of knee prostheses, by “Nexgen” and “other” prostheses types, subsequently showed in the “other” strata an inverse association between IFNγ and MTPM (β = −0.239; p < 0.001), whereas no association was observed in the “Nexgen” strata (β = −0.006; p = 0.938), independent of the covariates age, gender, time-to-blood sampling, and BMI. Exclusion of uncemented prosthesis, all belonging to the “other” strata, did not change the observed inverse association between IFNγ and MTPM.

This study is the first to indicate an inverse association between IFNγ and migration of knee prostheses. Nevertheless, the role of IFNγ in bone remodeling, which is relevant to implant fixation, has been investigated in several studies. Several in vitro studies showed that IFNγ either suppressed or enhanced osteoclastogenesis whereas another in vitro study showed that IFNγ positively stimulates osteoblastogenesis of human mesenchymal stem cells. Furthermore, an in vivo study, using different mouse models of bone loss showed that the net effect of IFNγ is that of stimulating bone resorption, whereas administration of IFNγ increased bone formation in wild-type mice and rescued ovariectomized mice from osteoporosis. Taken together, the role of IFNγ in the bone remodeling process is not completely clear, yet, based on our data, we hypothesize that IFNγ might have a beneficial effect on early migration. Whether this effect is due to either a decrease in osteoclastogenesis or an increase in osteoblastogenesis during initial fixation warrants further investigation. Furthermore, an in vitro study showed that titanium and polymethylmethacrylate (PMMA) bone cement particles have different effects on IFNγ signaling in osteoclast progenitor cells. Therefore, it could be that the role of IFNγ in early migration differs between type of fixation. Although interesting, we were not able to compare cemented and non-cemented TKA prostheses in our data set, as less than 10% of the measured prostheses were non-cemented. Moreover, the only non-cemented prosthesis included was the Interax prosthesis. Since the uncoated, non-cemented Interax prosthesis was shown to migrate excessively and have a three times higher revision rate compared to the cemented Interax prosthesis, we cannot exclude the role of type of fixation as possible confounder. Nevertheless, in this dataset when we excluded the uncemented prostheses from our analyses, the observed association between IFNγ and MTPM did not change. In this study, ex vivo blood stimulation with the TLR2 specific agonist Pam3Cys-SK4 was used rather than stimulation with wear particles. The use of wear particles may have given a more representative biological response, however, a study by Matthews et al. has shown that cells cultured with wear particles of different materials or sizes, secrete different types of functional inflammatory mediators. In this respect, Pam3Cys-SK4 responses have been shown to be easily reproduced and not to suffer from a possible contamination of endotoxins on wear particles. Furthermore, within literature, the possible role of TLR4 signaling activated by endotoxins of cell wall of gram-negative bacteria that reside on wear particles has been frequently addressed. For that matter, both TLR2 as TLR4 are found in tissue around loosened joint replacements. Additional studies are, therefore, necessary to investigate the role of TLR4
triggered innate immune responses in early migration of prostheses, as reflected by the capacity of blood cells to produce cytokine responses upon stimulation with lipopolysaccharides. These studies may confirm the here identified inverse association of MTPM and IFNγ also via TLR4 activation.

A drawback of this study is that not all blood samples were collected exactly 1 year after surgery. Cytokine levels were measured from blood samples collected 1–17 years postoperative. However, the observed association between cytokine levels, including IFNγ alone, and MTPM did not change when corrected for follow-up time as confounder. Therefore, it seems unlikely that the time to blood sampling has influenced the outcome.

Altogether our results indicate that patients with low levels of IFNγ upon stimulation of TLR2 are at higher risk of early migration of their prosthesis, particularly among patients who have received a prosthesis other than Nexgen. Eventually, our findings can be used to develop a preoperative prediction model for implant failure with specific focus on aseptic loosening. Such a prediction model will be important for patient’s follow-up (i.e., the frequency of postoperative radiographic control after TKA) as well as patient assessment in the preoperative state (i.e., postponing surgery if a reasonable risk for loosening is present).

AUTHORS’ CONTRIBUTIONS
All authors meet the criteria for authorship and all authors have read and approved the final submitted manuscript.

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REFERENCES


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