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Cellular Response To Prosthetic Wear Debris Differs In Rheumatoid Versus Non-Rheumatoid Arthritis

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Cellular Response to Prosthetic Wear Debris Differs in Rheumatoid Versus Non-Rheumatoid Arthritis

A Thesis Submitted to the
Yale University School of Medicine
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Medicine

by
Anant Vasudevan
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Abstract:
CELLULAR RESPONSE TO PROSTHETIC WEAR DEBRIS DIFFERS IN RHEUMATOID VERSUS NON-RHEUMATOID ARTHRTIS. Anant Vasudevan, Edward F. DiCarlo, Timothy Wright, Dan Chen, Mark. P Figgie, Steven R. Goldring, Lisa A. Mandl. Department of Rheumatology, Hospital for Special Surgery, Weill Cornell Medical College, New York, NY. (Sponsored by Carrie Swigart, Department of Orthopaedics and Rehabilitation, Yale University School of Medicine).

In the setting of inflammatory arthritis, the histopathologic reaction to foreign body wear debris generated from a failing arthroplasty has not been definitively characterized. This study examined whether patients with rheumatoid arthritis (RA) demonstrate different patterns of prosthetic wear or cellular responses to implant wear debris compared to patients without inflammatory joint disease. Thirty-eight patients who had a primary revision of a total elbow arthroplasty (TEA) for aseptic loosening between 1996 and 2008 were identified. Twenty-five had RA and 13 had no inflammatory arthritis. Clinical data, gross wear patterns of the removed prostheses, and histopathological analyses of peri-implant tissue were compared between RA and non-RA patients. Evaluation of the retrieved prostheses showed that conformational change of the humeral polyethylene bushing was associated with the generation of polyethylene and metal particles. The amount and type of wear debris in peri-prosthetic tissues was similar in RA and non-RA patients. RA patients not on anti-tumor necrosis factor (TNF) therapy exhibited a histologic pattern of interstitial and sheet-like lymphocytic infiltrates associated with a high plasma cell composition, which was different from the predominantly perivascular infiltrates with few plasma cells seen in non-RA patients (p-value = 0.04). RA patients on anti-TNF therapy showed a mixed perivascular and interstitial pattern of infiltrates with variable plasma cell composition. Based on this data, we propose that RA patients exhibit a distinct cellular response to implant wear debris compared with non-RA patients. This reaction was unrelated to differences in the type or amount of wear debris and was mitigated by anti-TNF therapy. These results suggest an intrinsic alteration in immunoregulation in RA and have implications for potential immunologic treatment of osteolysis in these patients.
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Introduction

*Rheumatoid Arthritis: Pathogenesis*

Rheumatoid arthritis (RA) is a common systemic autoimmune disorder, with an estimated population prevalence of 1%. It is characterized by persistent chronic inflammation, which classically destroys the tissue and bone of diarthrodial joints, but also has multiple systemic manifestations[1, 2]. The currently understood pathophysiology of RA is multifaceted, involving a genetically encoded predisposition towards an aberrant inflammatory response to both external and internal stimuli.

The post-translational modification known as citrullination facilitates an autoimmune pathway that is thought to be a key pathophysiologic mechanism of RA. The susceptibility to forming citrullinated proteins is most consistently linked with a genetic predisposition in the HLA-DRB1 gene[3]. This gene is responsible for producing MHC class II molecules involved in the presentation of peptides during the initiation of an immune response. However, aberrant alleles at this locus, especially HLA-DRB1*04, have been shown to create peptide-binding epitopes that preferentially bind citrullinated proteins generated from self-antigens [4, 5]. Recognition of these auto-antigens then precipitates a systemic autoimmune response. This response results in the formation of antibodies against citrullinated proteins that is clinically measured by the presence of anti-CCP (anti-cyclic citrullinated peptide) antibodies[3]. The current literature suggests that anti-CCP positivity and negativity represent two distinct subsets of rheumatoid arthritis[5-7].

Anti-CCP positive RA stems from both exogenous and endogenous factors, which stimulate an autoimmune response towards citrullinated proteins. For example,
citrullinated fibrinogen and vimentin have been used in the literature to elicit an autoimmune response through the HLA-DR1 shared epitope allele. Van de Woude et. al. showed that in the presence of HLA-DR1 and a smoking history, antibodies directed against vimentin conferred an odds ratio of 58 for RA compared to an odds ratio of 5.4 in the absence of these antibodies [6]. These ratios were much closer in patients with antibodies against fibrinogen[6]. Antibodies directed against citrullinated vimentin, fibrinogen, and alpha-enolase in particular show limited cross-reactivity. Antibodies against type II collagen appear to form in the absence of citrullination but again show limited reactivity against other antigens. Positivity toward each of these possible triggers appears to represent distinct subgroups of anti-CCP positive RA [5]. Such studies indicate that a combination of genetic risk factors, environmental risk factors such as smoking, and the presence of various citrullinated peptides together dictate the extent of an autoimmune response that is linked to rheumatoid arthritis.

However, where these peptides come from or what causes them to form is less clear. Snir et. al. demonstrated in RA patients that anti-CCP antibodies were present at higher levels in the synovial fluid compared to the serum when compared to tetanus toxoid controls, which were significantly higher in the serum. This point raises the possibility that anti-CCP antibodies are locally produced at the site of inflammation and rheumatologic disease [3, 5, 8]. Other studies have demonstrated ectopic lymphoid structures and the presence of clonal B cell aggregates in the synovial tissue of RA patients [3, 8]. Such findings are consistent with the production of anti-CCP antibodies within the RA synovium.
Furthermore, alpha-enolase is a glycolytic enzyme that has been found in the joints of patients with multiple types of inflammatory arthritis. Citrullinated alpha-enolase (CEP), however, has been associated more with RA, leading to an interest in anti-CEP antibodies as a more specific subset of anti-CCP antibodies for disease detection. One study indicated that anti-CEP antibodies have up to a 97% specificity for RA [9]. Mahdi et. al. recently published a study linking the presence of CEP to smoking. The mechanism of this process is unclear but smoking has also been independently associated with anti-CCP positivity[9]. The formation of anti-CEP antibodies is associated with the BRD 2 gene [9]. Studies also show that the concurrent presence of the HLA-DR1 shared epitope, a smoking history, and the PTPN22 gene is linked to anti-CEP positivity[3].

Comparatively less can be surmised about the pathogenesis of anti-CCP antibody negative RA. Though prior studies had indicated that the HLA-DR1*03 locus is associated with the autoantibody negative subset of RA, more recent studies have not found a statistically significant association[10]. A recent study looked at a genome-wide association of both HLA and non-HLA genes in RA patients with and without anti-CCP antibody positivity. The most significant difference between antibody positive and negative patients was on chromosome 6 in the HLA region. Of note, two non-HLA genes associated with anti-CCP antibody negative RA were identified: rs4305317 and rs6448119. The former is considered to be the best candidate gene with specificity for anti-CCP antibody negative RA [7]. However, before insight can be gained into this subset of RA, further studies corroborating the identification of this candidate gene are needed.
Rheumatoid Arthritis: A review of known immunopathology

As a disease entity whose pathogenesis involves autoantibody production by plasma cells, RA mediates inflammation by involving components of both the innate and adaptive immune system.

The initial phase of RA involves the formation of a pannus in the RA synovium. This formation consists of monocytes and fibroblasts whose infiltration into the synovium is facilitated by matrix metalloproteinases produced by the synovial lining cells. The pannus eventually becomes slightly vascularized and is covered by collagen. The tissue from which the pannus originates is thought to arise from type B synoviocytes[2].

Unlike other inflammatory diseases in which non-specific inflammation pervades the tissue, RA is often characterized by well-structured arrangements of inflammation. These well-developed ectopic germinal centers are present in the RA synovium, contain both T and B cells, and represent one of the most striking features of RA[1, 8]. Prior experiments have examined how T cells function in these germinal centers by comparing CD4+ T cell clones from the follicles of RA synovium to control CD4+ T cell clones from the peripheral blood [1]. Specifically, the output of cytokines was measured in response to fragments of RA synovium that were peripherally injected in a mouse model. The result was a multi-fold increase in the output of cytokines such as IFN-γ, IL-1β, and TNF-α in mice with follicular T cells. Interestingly, however, the production of these cytokines by CD4+ T cells was dependent on the presence of B cells, implicating B cells in T cell activation[1].
Furthermore, follicular dendritic cell networks comprise the aforementioned germinal centers in addition to T and B cells. Studies suggest that in the presence of these dendritic cell networks, activation-induced cytidine deaminase (AID) is also present[8]. AID is an enzyme required for class switching and hypermutation of immunoglobulins as they develop an affinity for particular antigens within CD21+ germinal centers. In addition, levels of AID within T or B cell aggregates correlated directly with anti-CCP antibody levels in the RA synovial tissue. This finding suggests that AID is associated with the formation of these antibodies, though a causative role has not been formally elucidated[8]. This study also corroborates that plasma cells in the germinal centers of RA patients do produce self-reacting antibodies as detected by anti-CCP antibody levels.

In addition, studies have documented the presence of IL-17 producing T cells (Th-17 cells) in RA synovial fluid. The quantitative levels of IL-17 were significantly greater in RA synovial fluid compared to both OA synovial fluid and RA peripheral blood samples [11]. Since IL-17 is thought to play a role in the inflammatory process leading to destruction of synovial tissue, bone, and cartilage, the finding that IL-17 producing CD4+ lymphocytes can be isolated from RA synovial tissues is significant[11]. In addition, observations suggest that IL-27 is involved in the differentiation of Th-17 cells while IFN-γ plays a role in Th-17 cell polarization, implicating two other cytokines in the potentiation of IL-17 activity. IL-23 has also been previously identified in the synovial tissue of RA patients but there is no conclusive evidence for its role in promoting Th-17 cell proliferation[11].

While CD4+ T lymphocytes have been traditionally considered the primary source of IL-17, recent studies suggest a role of the innate immune system in producing
IL-17. Specifically, mast cells in inflamed RA synovial tissue are often triggered by inflammatory stimuli such as complement, TNF-α, or autoantibodies to produce IL-17A[12]. Hueber et. al. also showed not only that mast cells have a likely role in RA pathogenesis, but also that the role of T lymphocytes in promoting inflammatory arthritis through mediation of IL-17 might be less than previously thought. In samples of RA synovial tissue, CD3+ cells (T cell marker) accounted for only 1-8% of IL-17A producing cells compared to 46-100% colocalization of MCT (mast cell marker) and IL-17A production [12]. This finding, however, does not imply that T lymphocytes in germinal centers do not mediate synovial inflammation through other pathways.

While many individual cytokines and chemokines have been implicated in downstream pathways that lead to the inflammation and tissue destruction of RA, recent studies have focused on TNF-α. TNF-α has been described for the past 25 years as a potential single mediator of RA, with specific attention paid to its presence in the synovial tissue, role in mediating both pro-inflammatory and anti-inflammatory cytokines, and even upregulation of TNF receptors in RA[13]. Clinical trials that initially compared anti-TNF therapy to placebo controls in the mid-1990’s demonstrated a 79% response rate with anti-TNF therapy as measured by a Paulus 20% response, compared to an 8% response in placebo controls[13, 14]. A Paulus 20% response is defined as a ≥20% improvement in at least 4 of the following 6 areas: joint tenderness, joint swelling, patient’s global assessment of improvement, physician’s global impression of improvement, morning stiffness, and ESR [15]. Given this clinical benefit, further studies have focused on the role of TNF-α in the pathogenesis of RA particularly because of its potential as a therapeutic target. Apart from its well-demonstrated role in mediating
inflammation, which in turn is associated with synovial tissue destruction, TNF-\(\alpha\) has been shown to affect angiogenesis, leukocyte trafficking, and hematologic cell lines, all of which can have systemic effects\[13\].

*Rheumatoid Arthritis: A review of treatment modalities*

As demonstrated in the previous sections, the pathogenesis of rheumatoid arthritis revolves around an aberrant immune response to self-antigens. That immune response involves extensive cytokine cascades, antibody production, and the local destruction of bone and tissue due to organized centers of inflammation. As a result, the basic principle of managing RA revolves around reducing inflammatory mediators. Though the approach is typically systemic, given the diffuse nature of inflammatory arthritis as well as the capacity of RA to produce extra-arthrodial disease, therapy has varying levels of specificity.

Prior to the mid 1990’s, the initial approach to treatment of RA involved steroids or NSAIDs until disease progression necessitated more potent anti-inflammatory medications known as DMARDs (disease-modifying anti-rheumatic drugs) \[2\]. However, subsequent studies indicated that early initiation of DMARDs in recently diagnosed RA patients resulted in improved outcomes at multiple endpoints including ESR levels, pain, and joint mobility \[2, 16\].

DMARDs have varying mechanisms of action but all mitigate inflammation either by preventing the proliferation and development of cells involved in inflammation or by neutralizing the inflammatory mediators produced by such cells. They can be broadly classified as either synthetic (most commonly methotrexate or leflunomide) or biological
(most commonly etanercept, adalimumab, or infliximab)[17]. Biological DMARDs are also referred to as anti-TNFα agents or biologics.

The American College of Rheumatology (ACR) recommendations as of 2008 suggested immediate initiation of methotrexate or leflunomide in all patients diagnosed with RA regardless of disease duration or severity[18]. Other less common synthetic DMARDs such as hydroxychloroquine or sulfasalazine were recommended if poor prognostic factors existed. Poor prognostic features were defined as including “functional limitations, extra-articular disease, RF positivity +/- anti-CCP positivity, and/or bony erosions by radiography”[18].

Per the ACR, use of biological DMARDs hinges more on the duration of disease. In patients with disease duration <6 months, the recommendation is for methotrexate along with a biologic only in patients with high disease activity. Patients with RA for longer than 6 months who failed prior methotrexate monotherapy, who have severe disease, or who have moderate disease but a poor prognosis were candidates for anti-TNFα agents[18].

Biologic DMARDs have been shown to be more efficacious when used as monotherapy compared to both methotrexate and placebo controls[19]. The relative effect of combination therapy is, however, unclear. Combining methotrexate with an anti-TNFα agent resulted in better clinical and functional outcomes when compared to monotherapy in some studies[17]. Subsequent studies indicate the improved efficacy conferred by combination therapy might be particularly true for patients who have failed methotrexate therapy as compared to methotrexate-naïve patients[19]. The current ACR recommendations acknowledge foremost the effectiveness of biologics, citing that they
are “efficacious in improving disease activity, function, and quality of life and/or retarding radiographic progression [of disease] when used alone, in combination with methotrexate, or in patients for whom treatment with DMARDs other than methotrexate led to an inadequate response” [18].

Furthermore, apart from lowering serum levels of pro-inflammatory cytokines, anti-TNFα agents can secondarily help in RA. Infliximab, which is a monoclonal antibody directed against TNFα, has been shown to lower CRP levels, thereby lowering levels of downstream complement activation. Complement activation has been previously linked to the pathogenesis of RA in animal models [20].

When considering adverse events associated with anti-TNF therapy, the most common concern is infection [21]. Infliximab, in particular, has been associated with a higher incidence of severe infection [19]. Multiple studies have raised the possibility of etanercept being linked to a higher incidence of malignancy, including lymphoma and skin cancer. However, no statistically significant association exists, including in a meta-analysis of more than 3300 patients [19, 21, 22]. There is limited evidence from clinical trials suggesting that newer biologics such as abatacept and rituximab might have more favorable safety profiles, though this is far from conclusive [21].

**Peri-Implant Osteolysis**

Despite the reviewed understanding of the natural history of RA as well as management strategies that target its immunologic pathophysiology, the disease and its optimal management have not been fully characterized in patients with arthroplasties. When joint destruction related to the severity of disease necessitates a prosthetic implant,
RA patients not only have to deal with their underlying inflammatory arthritis but also the body’s response to an implant.

Orthopedic interventions are relatively common in RA patients. Of 183 RA patients who were followed for 16-20 years in a prospective study, 58% underwent some form of orthopedic surgery and 24% had a joint replacement. Arthroplasties of all major joints were noted with hips and knees being the most common, but non-weight bearing joints such as elbows, shoulders, wrists, and fingers together represented 36% of the arthroplasties performed [23]. A prosthetic joint has multiple components. It typically consists of two metal stems that are inserted into each of the two bones forming a joint. These metal stems are often cemented in place and are joined with metal axles, using polyethylene bushings as a lubricating surface.

From a biomechanical perspective, the polyethylene bushings absorb great amounts of impact and are vulnerable to deterioration over time. In elbow arthroplasties, for example, Lee et. al. found that the polyethylene bushings had to be replaced at an average of 7.9 years[24]. Goldberg et. al. also found that the ulnar and humeral polyethylene bushings found in total elbow arthroplasties are the weakest components of the device[25]. As the implant is in place for an extended period of time, the polyethylene surface is slowly deformed leading to loss of microscopic pieces of polyethylene into the tissue surrounding the arthroplasty. If enough of the polyethylene lubricating surface is deformed, the metal components of the implant eventually articulate causing pieces of metal debris to also spread into the peri-prosthetic tissue.

Numerous studies have demonstrated that both polyethylene and titanium metal particles in the peri-prosthetic tissue are linked to osteolysis, or loss of peri-implant bone,
which in turn causes aseptic loosening of the arthroplasty. Initial models suggested three potential mechanisms for wear debris-induced inflammation that bring about destruction of bone[26].

First, macrophages activated by the phagocytosis of particulate debris have been shown to generate nitric oxide. Such free radicals in turn worsen inflammation in the joint tissue and facilitate the activation of osteoclastic differentiation and enzymatic activation[26]. Initial studies indicated that titanium alloy had the largest effect in stimulating nitrite production in a mouse model, likely through upregulation of inducible nitric oxide synthase. Pure titanium metal and polymethylmethacrylate showed slightly lower levels of nitrite production[27].

Second, Wang et. al. demonstrated that exposure of bone marrow cells to foreign body wear debris over time causes a decrease in mesenchymal stem cells’ (MSC) ability to differentiate into osteoblasts. The mechanism through which titanium particles in particular suppress osteoblastic differentiation appears to hinge on suppression of the BSP gene[26]. Third, titanium particles have also been linked to promoting apoptosis of MSC’s. Mechanisms of reduced MSC viability include p53-mediated apoptosis and activation of death receptors by TNFα[26].

While many of the previously noted studies suggest a significant role for titanium metal in bringing about osteolysis, polyethylene has been extensively linked to aseptic loosening as well. Numerous studies suggest that the tissue reaction to polyethylene debris depends on the size and manufacturing properties such as cross-linking[28]. The mechanism of polyethylene-induced osteolysis is postulated to be similar to titanium metal particles in that macrophages activated by the phagocytosis of particles bring about
a release of pro-inflammatory cytokines, including TNFα. These cytokines in turn upregulate matrix metalloproteinases as well as RANK and RANK ligand, which directly increase osteoclastic differentiation and proliferation leading to osteolysis[28, 29]. At the same time, bone formation is uncoupled from resorption such that apoptotic pathways preferentially promote the survival of osteoclasts and death of immature osteoblasts, largely through the action of TNFα [28, 30, 31]. The dose-dependent preference of apoptotic pathways for immature over mature osteoblast lineages has been specifically demonstrated in vitro[31].

Although the role of the innate immune system in responding to foreign body wear debris has been well described, the role of the adaptive immune system is less certain. Willert et. al. described the presence of a lymphocytic infiltrate in the peri-prosthetic tissue of patients with metal-on-metal hip implants that had resulted in aseptic loosening[32]. Goldberg et. al. examined the peri-articular tissue taken during revision of a total elbow arthroplasty for aseptic loosening due to osteolysis. While this study noted a robust giant cell and histiocytic response, 69% showed a minimal lymphocytic response and 12% showed a significant lymphocytic response[25]. Hallab et. al. showed a Th1 lymphocytic response was detected in response to a metal challenge test in patients with total hip arthroplasties. An increase in IFN-γ, which is released by Th1 lymphocytes and is associated with osteoclast activity, supports this claim. The emergence of a subset of T lymphocytes indicates a level of specificity consistent with an adaptive immune response to metallic debris[33]. While some studies have also postulated how RANKL expression on the surface of T cells is sensitive to TNFα and might also promote osteolysis, other studies have shown no osteolysis in lymphocyte-deficient mice[30, 34].
Purpose of Present Study

Peri-implant osteolysis and associated loss of fixation is the major reason total joint replacements fail. The progressive loss of bone adjacent to the implant has been attributed to a granulomatous inflammatory reaction induced by particulate implant wear debris at the bone-implant interface [35]. Patients frequently exhibit dramatic differences in the rate of peri-implant bone loss, and it is unclear whether this reflects differences in the properties or amount of wear debris, differential patterns of biomechanical failure, or differences in individual host immune response.

This distinction regarding the underlying mechanism driving aseptic loosening is particularly critical in patients who are already susceptible to an overblown inflammatory response, as occurs in RA. RA patients with arthroplasties respond to both the baseline inflammatory properties of particulate debris, and the organized inflammation and joint destruction characteristic of this inflammatory arthritis.

Studies by Caplan and coworkers noted that coal miners with RA who were exposed to silica containing coal dust were uniquely prone to the development of large pulmonary granulomas (“Caplan’s nodules”) [36]. They speculated that this differential response to an inorganic particulate reflected an intrinsic alteration in immunoregulation among RA patients. Similarly, RA patients may exhibit a unique immune response to the inorganic wear particles generated by prosthetic implants, which might lead to different failure mechanisms compared with non-RA patients.

Kaufman et. al. reported on a series of patients undergoing revision surgery for aseptic failure after TEA [37]. They did not note a difference in the cellular features of the peri-implant tissue reaction in patients with or without inflammatory arthritis. More
recently, Goldberg et. al. evaluated the tissue reaction accompanying failed, semi-constrained, Coonrad-Morrey elbow implants at primary revision in sixteen patients, nine of whom had inflammatory arthritis [25]. Similarly, the peri-prosthetic tissue showed no evidence of a differing cellular reaction on the basis of underlying diagnosis. In contrast, Goldring et. al. found that among a similar group of patients, lymphoplasmacytic inflammation was found exclusively in RA patients [38]. Factors responsible for these contradictory findings could include differences in the composition and types of devices, differences in patient populations, and differences in treatment.

Aims of Study

The present study was undertaken to compare cellular features of the peri-implant tissue response among patients with and without RA undergoing revision of a TEA due to aseptic loosening, and to ascertain the relationship with underlying disease, patterns of gross device wear, and the amount and type of particulate debris.
Patients and Methods

Identification of Patients

Surgical specimens from all primary TEA revisions performed between 1996 and 2008 for aseptic loosening were identified from the database of the Department of Pathology and Laboratory Medicine at the Hospital for Special Surgery (HSS). Given that the elbow is a non-weight bearing joint, this population is not predominated by degenerative joint disease and is, therefore, enriched for RA patients. Implant components corresponding to the identified cases were recovered through the Implant Retrieval Registry in the Hospital’s Department of Biomechanics. The patients’ primary diagnosis, gender, race, age at primary TEA, age at revision TEA, pre-revision medical treatment, pre-revision flexion-extension range of motion, dominant hand, as well as height and weight at the time of revision were obtained from medical records. Non-RA patients include non-inflammatory arthritis patients with a history of OA or trauma. RA patients were labeled treated or untreated based on pre-operative usage of a TNF-inhibitor. When stratifying patients on this basis, those in whom this data was unavailable were excluded from analysis. Duration of implantation was defined as the number of years between the original TEA and primary revision TEA. A short duration of implantation was <4 yrs; a long duration of implantation was ≥ 4 yrs. HSS Institutional Review Board approval was obtained for this study.

Device Evaluation

As available, the retrieved humeral and ulnar components, humeral polyethylene bushing (Fig. 1A), and axle were evaluated for evidence of wear and surface damage.
Due to the limited number of metal components (Fig. 1B), no information was collected on metal loss.

A previously developed subjective scoring method was used to assess seven modes of polyethylene damage [39]. Of the seven modes, conformational change, due either to deformation or material loss, was considered the most accurate and functionally relevant metric of biomechanical damage. Grades of none, mild, moderate or severe corresponded with 0, <10%, 10-50%, and >50% of the articulating surface area being visually thinner than the non-articulating surface. However, this scoring system was insufficient to estimate the extent of wear in instances of penetrating focal damage. Focal wear was measured separately as the proportion of thinning in a localized area between the inner and outer bushing diameters compared to a non-deformed region. Focal wear was graded as none, mild, moderate, or severe corresponding to the diameter being 0, <10%, 10-50%, and >50% thinner compared with the non-deformed surface. A device’s final grade was the highest qualifying score on the basis of either surface area damaged or focal wear.

All grading was done under a stereomicroscope while blinded to both the underlying diagnosis and tissue histology. Grading was performed independently by two readers (AV and DC). Differences were resolved by consensus.

**Histopathology**

Hematoxylin and eosin stained samples of peri-prosthetic tissue were evaluated for the type and size of particulate material, as well as the presence, type, location, and intensity
of inflammatory infiltrates. This analysis is based on a modification of a similar analysis published elsewhere [40].

Polyethylene debris particles were classified as either small (<500 µm) or large (≥500 µm) based on the largest dimension (Fig. 1C). Metal particles were not stratified by size, as they were uniformly small particles (~10 µm) with little heterogeneity on the basis of visual identification. The distribution of polyethylene debris was defined as low prevalence or high prevalence. Low prevalence was defined as wear particles seen in ≤50% of the area of at least 2 histologic fields viewed at 10x magnification. For metallic debris, which was less common, low prevalence was defined as particles seen in ≤10% of the area of at least 2 histologic fields viewed at 10x magnification.

In describing the peri-prosthetic tissue reaction, monocytes and giant cells were defined as low prevalence if they comprised ≤ 50% of the cellular infiltrate on two separate 10x fields, and high prevalence if they comprised >50%. A lymphocytic aggregate was defined as a group of ≥10 lymphocytes visualized on a 5x field. Perivascular infiltrates surrounded a discernable vascular structure (Fig. 1D). Focal interstitial infiltrates were defined as aggregates of lymphocytes in the absence of a discernable vascular structure (Fig. 1E). Sheet-like aggregates were defined as a diffuse interstitial pattern of lymphocytic infiltrates throughout the entire tissue section (Fig. 1F). Focal interstitial and sheet-like aggregates represent two degrees of intensity of interstitial lymphocytic inflammation. When evaluating plasma cells within lymphocytic aggregates, low prevalence of plasma cells was defined as comprising ≤ 10% of the lymphocytic aggregate, and high prevalence >10% of the aggregate in two separate 10x fields.
To confirm that our histologic identification of cell types was correct, immunohistochemistry was performed on tissue from a representative subset of cases. T- and B-lymphocytes were identified by using anti-CD3 and anti-CD20 antibodies, respectively, and plasma cells by using anti-Kappa and anti-Lambda antibodies.

Figure 1. A.) Humeral polyethylene bushing showing focal, severe conformational change (thin arrow). Moderate conformational change present on the opposite end of bushing (thick arrow). B.) Moderate metal loss on the proximal portion of the humeral stem (white arrow). C.) Small particulate polyethylene of high prevalence shown in polarized light at 10x. D.) Perivascular lymphocytic inflammation (40x) E.) Interstitial lymphocytic inflammation (40x) with visible plasma cells (thin arrow). Metallic debris being phagocytosed by foreign body giant cells (thick arrow). F.) Sheet-like lymphocytic infiltration with widespread plasma cells (thick arrow) at 40x. Metallic debris present across entire field (white star).
**Osteolysis**

Available anterior-posterior (AP) and lateral pre-operative radiographs were evaluated for the presence of osteolysis based on a previously used scoring system [25]. The humeral and ulnar stems of the prosthetic were divided into 4 zones each and examined for the presence of radiolucencies at both the cement-prosthesis and cement-bone interface.

**Statistical Analysis**

Fisher’s Exact test was used to compare all categorical variables. Sample size made this a more high fidelity test than Chi-squared. T-tests were performed on continuous data. A kappa analysis was performed to establish inter- and intra-rater reliability of the qualitative histologic scoring.

**Contributions to Present Study**

Primary responsibility for all data collection, compilation, and analysis was held by Anant Vasudevan. Dr. Lisa Mandl was the principal investigator who oversaw and contributed to all progress in data collection, analysis, and communication of results. Anant Vasudevan, Dr. Mandl, and Dr. Goldring shared responsibility for study design, analysis of data, and interpretation of results. In examining devices, Dan Chen and Anant Vasudevan shared equal responsibility in grading all aspects of implant wear. Dr. Wright aided in the design of parameters through which to assess devices and provided opinions as needed. In examining histology samples, Dr. DiCarlo provided expert qualitative impressions of both particle burden and the extent of the inflammatory tissue reaction to
wear debris. Anant Vasudevan and Dr. DiCarlo examined all histopathology slides and collected all data together. Immunohistochemical analysis of histology samples was performed commercially. The chart review was performed by Anant Vasudevan. In assessing osteolysis, Anant Vasudevan helped assemble the parameters for evaluating radiographs, and Dr. Figgie, an orthopedic surgeon, provided expert reads of films. Statistical analysis was performed by Anant Vasudevan except the kappa analysis for inter/intra-rater reliability, which was done by Dr. Stephen Lyman of the HSS Biostatistical Core. All authors contributed to revising the Methods, Results, and Discussion sections of this manuscript.
Results

Profile of Patients

Of 88 cases, 11 were excluded because of infection. Of the remaining 77 cases, 39 had inadequate tissue for review. Complete medical records were available in 36 of the remaining 38 patients; two had only the operative record and partial medical records.

Twenty-five of the 38 patients had RA (66%), 7 had OA (18%), and 6 had a history of traumatic elbow injury without underlying inflammatory arthritis (16%). Of the 25 RA patients, 24 had pre-operative medication data. Ten RA patients were on a TNF inhibitor pre-operatively (etanercept in all cases). The remaining 14 RA patients were not on a TNF inhibitor pre-operatively. None of the non-RA patients were pre-operatively on a TNF inhibitor. 4 patients (3 RA and 1 non-RA) underwent revision surgery prior to FDA approval of etanercept.

Of all RA patients, 91% were Caucasian, 84% were women, and 95% were right-handed compared to 100%, 87%, and 100% respectively in non-RA patients. The right elbow was the operative site in just under half of both groups. Thirteen percent of RA patients and 33% of non-RA patients had a BMI >30 (p = 0.19). The average pre-operative range of motion was 112° (0 to 210°) for RA patients and 109° (60 to 130°) for non-RA patients (p = 0.85). RA patients were younger at the time of original implant surgery (p=0.0003). The average duration of implantation was 8.9 yrs for all RA patients and 5.3 yrs for non-RA patients (p = 0.06) (Table 1).

Untreated RA patients had a longer time to primary revision than treated RA patients (10.9 vs. 5.8 yrs, p = 0.04) or non-RA patients (10.9 vs. 5.3 yrs, p = 0.01). There
was no difference in the duration of implantation between non-RA patients and treated RA patients (5.8 vs. 5.3 yrs; p = 0.77).

<table>
<thead>
<tr>
<th></th>
<th>RA: 25</th>
<th>Non-RA: 13 (7OA, 6 Trauma)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>84%</td>
<td>87%</td>
<td>1.0</td>
</tr>
<tr>
<td>Race (N = 23)</td>
<td></td>
<td>(N = 13)</td>
<td>1.0</td>
</tr>
<tr>
<td>Caucasian</td>
<td>91.3%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>4.3%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>4.3%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Avg. Age at Original Implant</td>
<td>50.8 ± 12.3 yrs (N = 24)</td>
<td>66.1 ± 8.1 yrs</td>
<td>0.0003</td>
</tr>
<tr>
<td>Avg. Age at First Revision</td>
<td>58.3 ± 15.0 yrs</td>
<td>71.4 ± 6.8 yrs</td>
<td>0.005</td>
</tr>
<tr>
<td>Duration of Implantation</td>
<td>8.9 ± 5.8 yrs</td>
<td>5.3 ± 4.3 yrs</td>
<td>0.06</td>
</tr>
<tr>
<td>Range of Motion (Flex-Ext)</td>
<td>112.2° (N = 20)</td>
<td>108.9° (N = 8)</td>
<td>0.85</td>
</tr>
<tr>
<td>Index Elbow</td>
<td>44% Right Elbow</td>
<td>46% Right Elbow</td>
<td>1.0</td>
</tr>
<tr>
<td>Dominant Hand</td>
<td>95% right-handed (N = 19)</td>
<td>100% right-handed (N = 13)</td>
<td>1.0</td>
</tr>
<tr>
<td>BMI (&lt; 30 vs. ≥ 30)</td>
<td>(N = 24)</td>
<td>(N = 12)</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>18.5 – 24.9</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>25.0 – 29.9</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>≥ 30.0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>DMARDS:</td>
<td>(N = 24)</td>
<td>(N = 12)</td>
<td></td>
</tr>
<tr>
<td>Etanercept</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>+ Methotrexate</td>
<td>3</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>+ Imuran</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Bisphosphonates</td>
<td>3</td>
<td>4</td>
<td>0.40</td>
</tr>
<tr>
<td>Prednisone</td>
<td>6</td>
<td>0</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Table 1. Descriptive statistics on 38 TEA patients. 25 RA and 13 non-RA patients used for each variable unless otherwise indicated.
Biomechanical Wear

Of the 38 patients in this study, 24 had semi-constrained retrieved implants available for analysis. Twelve of the implants were Osteonics Linked Semi-constrained replacements manufactured by Osteonics, 7 were Coonrad-Morrey implants manufactured by Zimmer, 2 were Solar devices manufactured by Stryker, 2 were Triaxial prostheses manufactured by Johnson and Johnson, and 1 was a custom device. All devices had humeral polyethylene bushings. Metal components were available for 15, of which 3 contained both humeral and ulnar components, 7 contained only the humeral component, and 5 contained only the ulnar component. Five of the axle-containing devices had no axle available for analysis. All metal components were titanium.

Conformational changes in the 24 humeral polyethylene bushings were mild in 10, moderate in 3, and severe in 11. Moderate changes were qualitatively much closer to severe than mild, so moderate and severe categories were grouped for statistical analysis. No difference in severity of conformational changes were noted between Coonrad-Morrey implants and other designs (p = 0.08). More severe conformational change was associated with a longer duration of implantation of >4 years (p = 0.01). Conformational change showed no association with underlying diagnosis (RA vs. non-RA, p = 1.0), or with use of TNF inhibitors (p = 1.0) (Fig. 2A), regardless of whether the 3 instances of moderate changes were excluded.

Histopathologic Analysis

a) Particulate Debris

Polyethylene and metal particles were observed in varying quantities in the peri-prosthetic tissue reaction. Increased polyethylene particle prevalence (p = 0.01) and size
(p = 0.005) and increased metal particle prevalence (p=0.05) were all associated with more severe conformational change (Fig. 2B-D).
Increased polyethylene particle prevalence ($p = 0.06$) and increased polyethylene particle size ($p = 0.03$) were associated with duration of implantation > 4 yrs. Metal particle prevalence was not associated with duration of implantation ($p = 0.15$).

No difference was found in the prevalence or size of polyethylene particles among non-RA patients, treated RA patients, or untreated RA patients ($p \geq 0.65$ for all comparisons) (Fig. 3A-B). Similarly, no difference existed in the prevalence of metal particles among these three groups ($p \geq 0.68$) (Fig. 3C).

Figure 3. Prevalence of polyethylene particles (A), size of polyethylene particles (B), and prevalence of metal particles (C) did not vary with underlying diagnosis or anti-TNF therapy.
b) Tissue Reaction:

A higher prevalence of foreign body giant cells was associated with a higher prevalence of polyethylene particles (p < 0.001), larger polyethylene particles (p = 0.004), and higher prevalence of metal particles (p = 0.04). There were no significant differences in the prevalence of foreign body giant cells (p = 0.73) between non-RA patients and RA patients. This finding did not change when RA patients were stratified by use of anti-TNF therapy (p = 1.0).

No association was found between the prevalence of monocytes and the prevalence of polyethylene particles (p = 0.24), size of polyethylene particles (p = 0.25), or prevalence of metal particles (p = 1.0). There were no significant differences in the prevalence of monocytes (p = 0.12) between non-RA patients and RA patients. This finding did not change when RA patients were stratified by use of anti-TNF therapy (p = 1.0).

The presence of any lymphocytic aggregates was associated with lower metal particle prevalence (p = 0.005) but not with polyethylene particle prevalence (p = 0.14) or size (p = 0.32). An increased plasma cell prevalence within lymphocytic aggregates was associated with widespread metal particles (p = 0.049) but was not significantly associated with polyethylene prevalence (p = 0.36) or size (p = 0.40).

c) Extent of Inflammatory Reaction:

In the presence of any type of wear debris, untreated RA patients showed an interstitial pattern of lymphocytic aggregation as opposed to the predominantly perivascular pattern seen in non-RA patients (p = 0.04). Treated RA patients showed a mixed pattern of perivascular and interstitial lymphocytic infiltrates intermediate between
non-RA patients and untreated RA patients (Fig. 4A). 50% of untreated RA patients also showed a sheet-like intensity of lymphocytic infiltrates compared to none of the non-RA patients (p = 0.09). Treated RA patients showed a mixed intensity of lymphocytic infiltrates with 22% being diffuse, sheet-like infiltrates.

A similar pattern of lymphocytic infiltration was seen when considering only patients with small or few wear particles of any type. The majority of these patients had ≤10% plasma cells in the lymphocytic aggregates, with no significant difference when comparing underlying diagnosis, treatment, or particle composition.

Among patients with a high prevalence of polyethylene debris, including small and large particles, all untreated RA patients showed lymphocytic infiltrates with an interstitial or diffuse sheet-like appearance, associated with a high prevalence of plasma cells. This pattern was present in only half of treated RA patients. Only one non-RA patient exhibited this pattern of polyethylene debris, and in this patient the lymphocytic aggregates were perivascular and associated with a low prevalence of plasma cells (p =0.33) (Fig. 4B). The cellular patterns were similar in tissue sections containing predominantly large non-phagocytosable polyethylene particles (Fig. 4C). Again, all untreated RA patients had a high prevalence of plasma cells in lymphocytic infiltrates compared with none of the non-RA patients (p = 0.07). Only one-third of treated RA patients exhibited this pattern.

In the presence of a high prevalence of metal debris, untreated RA patients had exclusively lymphocytic aggregates with an interstitial or sheet-like appearance that were associated with a high plasma cell prevalence (Fig. 4D). Unlike the histologic findings associated with polyethylene, 40% of the non-RA patients had lymphocytic aggregates
with high plasma cell prevalence; however, unlike the RA patients, the plasma cells were associated with focal interstitial or perivascular lymphocytic aggregates. Treated RA patients had a prevalence of plasma cells similar to the non-RA patients (p = 1.0).

Figure 4. A.) Untreated RA patients show predominantly interstitial and sheet-like lymphocytic aggregates in response to foreign body wear debris. This is significantly different from non-RA patients. Treated RA patients show a mix of perivascular and interstitial aggregates in between non-RA and untreated RA patients. In patients with a high prevalence of polyethylene (B) or large particulate polyethylene (C), non-RA patients show exclusively a low plasma cell prevalence within lymphocytic aggregates. Untreated RA patients show exclusively high plasma cell prevalence. D.) In patients with a high prevalence of metal particles, untreated RA patients show exclusively high plasma cell prevalence within lymphocytic aggregates. ≥ 50% of treated RA patients and non-RA patients showed a low plasma cell prevalence.
d) Immunohistochemistry

A representative convenience sample of peri-prosthetic tissue from 2 non-RA cases, 3 treated RA cases, and 3 untreated RA cases was selected for immunohistochemical staining. T- and B-lymphocytes were identified by using anti-CD3 and anti-CD20 antibodies, respectively, and plasma cells by using anti-Kappa and anti-Lambda antibodies. Repeat evaluation for the presence, type, location, and intensity of inflammatory infiltrates using cells identified via immunohistochemistry showed identical results to the reviews based on visual identification of cell types.

Radiographic Analysis

Of the 38 patients in this study, 18 had lateral radiographs and 17 of these 18 had AP radiographs available for review. These 18 patients included 5 non-RA, 3 treated RA, and 10 untreated RA patients.

At the cement-prosthesis interface, none of the RA patients had radiolucencies, regardless of treatment. One of five non-RA patients showed radiolucencies. At the cement-bone interface, 9 of 18 patients showed radiolucencies, including 2 non-RA, 2 treated RA and 5 untreated RA patients. There was no association between the presence of radiolucencies and treatment with etanercept, among RA patients (p = 1.0). Similarly, there was no difference in the presence of radiolucencies between non-RA and RA patients (p = 1.0).
Inter/Intra-rater Reliability of Statistical Methods

7 of 24 devices and 10 of 38 slides were randomly selected for kappa analysis. The inter-rater kappa values were 0.84, 1.0, 0.58, 0.62, 0.29, and 1.0, and the intra-rater analysis values were 0.63, 1.0, 0.74, 0.74, 0.55, and 1.0 for location of lymphocytic infiltrate, plasma cell prevalence, polyethylene prevalence, polyethylene size, metal prevalence, and deformation of the humeral polyethylene bushing respectively.
**Discussion**

Our results show that conformational change in the polyethylene bushing is strongly associated with large particulate polyethylene debris and a high prevalence of metal and polyethylene particles, confirming that conformational change in the bushing is due to loss of material, not just deformation. Of note, no difference was found between RA and non-RA patients in terms of the severity of device deformation, the composition or magnitude of wear debris, or the average pre-operative range of motion, suggesting that differences in cellular and tissue reactions cannot be attributed to these factors. The only positive association with less conformational change and wear debris was having a primary TEA revised <4 years after implantation, regardless of underlying diagnosis.

We found an increase in the intensity of lymphocytic aggregates among untreated RA patients, which correlated with the presence of metal and polyethylene particles. This finding suggests activation of the adaptive immune system in these patients. Analysis of the pattern of prosthetic wear revealed that the ulnar and humeral polyethylene bushings exhibited extensive wear and fragmentation, including metal particle formation related to unintended metal-on-metal wear. Previous studies reported the presence of an inflammatory cell infiltrate and multinucleated giant cells with regions of fibrosis as well as focal necrosis associated with extra- and intracellular metallic and polymeric particles within histiocytes [25, 41]. Only a minimal lymphocytic reaction was found in most cases, and no difference in cellular characteristics was reported between patients with or without inflammatory arthritis. Our study suggests that while no increase occurred in the presence of lymphoplasmacytic infiltrates in patients with inflammatory
arthritis, the degree of plasma cell infiltrates was significantly increased among those with diffuse lymphocytic aggregates.

The presence of plasma cells associated with metal wear debris is a characteristic histologic feature in patients undergoing revision surgery after metal-on-metal hip resurfacing [42, 43]. Diffuse aggregates of lymphocytes and plasma cells are associated with regions of tissue necrosis and extensive fibrin deposition. The reaction is thought to represent a hypersensitivity to the metal wear products. These findings differ substantially from those in our series and other TEA series [25, 41], and may represent a distinct clinical entity in a weight bearing joint. However, most of the patients on anti-TNF therapy in our study demonstrated a similar, high prevalence of plasma cells only in tissue sites with high metal debris prevalence, suggesting that metal debris may initiate the plasmacytic response. The presence of lymphocytic aggregates, however, was significantly associated with lower metal particle prevalence. This suggests that while metal might specifically bring about a plasmacytic response, polyethylene may be driving the broader lymphocytic response in these patients. Though no definitive correlation exists between polyethylene and a robust lymphocytic response, prior studies have demonstrated lymphocytic infiltrates in response to wear debris even from arthroplasties that are not exclusively metal-on-metal [25]. Another possibility is that metal particles in the nanometer range, which have been previously described particularly in metal-on-metal implants and are not counted through visual identification, could cause an underestimation of the true metal particle burden and its contribution to a lymphocytic response [44].
Fujishiro et al. recently reviewed a large series of patients undergoing revision surgery after total hip replacement for non-metal-on-metal implants [45]. Their series was restricted to patients without known inflammatory arthritis, and included patients undergoing revision surgery for septic joints. They noted lymphocytic infiltrates in half of the patients with aseptic loosening, 62% of whom demonstrated a diffuse pattern of lymphocytic infiltration. They noted that the diffuse pattern was most commonly associated with regions of metal wear accumulation. Only 7% of aseptic cases had plasma cells associated with the lymphocytic aggregates. Their detection of plasma cells in a low number of non-RA patients is similar to our findings, and contrasts with the high prevalence of these cells in our RA patients.

The present study’s findings confirm earlier observations in RA patients undergoing revision surgery for failed total knee replacement [38]. Although we detected the presence of lymphocytic infiltrates in the tissue from both RA and non-RA patients, the pattern of tissue distribution and extent of the infiltrates differed substantially between the two patient subsets. The infiltrates in non-RA patients were focal with predominantly perivascular localization and few plasma cells. In contrast, in RA patients the infiltrates exhibited both a perivascular and interstitial pattern of distribution and were often organized into sheet-like infiltrates with a high proportion of plasma cells. High polyethylene particle prevalence and large polyethylene particle size were associated with a high plasma cell prevalence within lymphocytic aggregates in RA patients. In contrast, no non-RA patients with many or large polyethylene particles in the peri-implant tissue had lymphocytic aggregates with a high plasma cell prevalence. The prevalence of plasma cells in treated RA patients fell between these two groups, suggesting that the
reduction in systemic and tissue inflammation associated with anti-TNF therapy results in attenuation of the pattern of cellular responses observed in RA patients.

Our results provide evidence that patients with RA exhibit a differential cellular reaction to inorganic metallic and polyethylene debris compared to patients without RA. The tissue reaction in the non-RA patients exhibited features of a non-immune granuloma. Lymphocytic infiltrates were scattered within the tissue and limited primarily to a perivascular location. In the untreated RA patients, the presence of more generalized lymphocytic infiltrates containing abundant plasma cells are indicative of participation of cellular components of both the innate and adaptive immune system, a feature characteristic of an “immune granuloma.” These findings recapitulate the observations of Caplan and coworkers who described the presence of atypical pulmonary nodules in a series of coal miners [46]. Histopathologic analysis of those nodules revealed the presence of immune granulomas with extensive lymphocytic infiltration with T and B cells. Importantly, these lesions were absent in miners without RA or in RA patients without exposure to coal dust, leading to the hypothesis that these findings reflected an aberrant immune response to the inorganic components of the coal dust in the RA patients.

The nature of the underlying immunologic mechanisms by which inorganic particles initiate an inflammatory reaction with cellular features of an immune granulomatous response remains speculative. Importantly, this reaction in both peri-implant tissues and “Caplan’s nodules” develops in the absence of articular cartilage. Cartilage matrix components have been implicated as potential immunogens in the pathogenesis of RA synovitis; our observations provide evidence that cartilage is not
playing a role in the immune response [47]. The association of anti-TNF therapy with diminished cellular features of the tissue reaction parallels its known effects in reducing inflammatory cell infiltrates in RA synovium [48].

In addition, there was no difference in the presence of radiolucencies between patients treated and not treated with etanercept. While limited by a small sample size and the unavailability of three-dimensional imaging, these findings are consistent with other studies, which have not found anti-TNF medications to be protective against osteolysis [49].

Our data show a trend towards the TEA lasting longer in RA patients compared with non-RA patients. This could be due to lower demands placed on the joint replacement by RA patients. However, treated RA patients show a length of implantation similar to non-RA patients and shorter than untreated RA patients. Users of TNF-inhibitors may have had better-controlled disease, enabling them to resume normal function with higher forces across the joint, accelerating prosthesis loosening.

Finally, the inter-rater and intra-rater reliability testing of important qualitative variables in our study helps support the integrity of our dataset. The acceptable kappa values provide evidence that our scoring methods are reproducible, accurate, and unaffected by the personal bias of a scorer directly involved in our study. Having this confidence is critical in evaluating the raw data presented in this paper, especially given the subjective nature of determining the extent of device wear and recognizing both wear debris particles and immunologic cell morphology in peri-prosthetic tissue. However, strict, detailed, and previously vetted criteria were used as the basis for all qualitative evaluation, and the dependability of the results was confirmed by independent scorers.
In summary, we provide evidence that patients with RA exhibit a differential cellular response to prosthetic wear debris compared with non-RA patients, and that this reaction is mitigated by the use of anti-TNF inhibitors. The immune tissue reaction is similar to that observed in RA patients exposed to silica, another inorganic particle, and specifically consistent with earlier findings of Goldring et. al [38]. Of note, this reaction develops in the absence of articular cartilage, which has been implicated in the immunopathogenesis of RA synovitis [47, 50]. These results provide evidence of a pathogenic activation of the adaptive immune response in RA and could have implications for the treatment of peri-implant osteolysis.

Limitations

Limitations of our study include the small sample size and retrospective data collection. Data were collected cross-sectionally from the time of revision surgery, making it impossible to assign causation. Histopathologic analysis was also limited by the number of available slides. Etanercept was FDA approved in 1998. Patients with more severe RA operated on after 1998 may have been more likely to receive a TNF inhibitor, a potential systematic bias. However, the fact that anti-TNF therapy was not readily available until early 1999 prevented any confounding by indication for the first three years of our cohort. Inter-rater and intra-rater reliability testing was performed for all key variables in this study except those associated with evaluation of radiographs. The previously described scoring system by Goldberg et. al. was specifically designed for a TEA and explicit in terms of device evaluation. As a result, only one read of films by an expert orthopedic surgeon was performed, but this is a potential limitation of our study.
Future Directions

Our study helps provide further evidence of an intrinsically altered immune response in patients with RA. Validating these results with a larger cohort of patients would be an important follow-up study. Increasing the sample size would not only help increase the statistical power of the results, but also allow for further stratification of patients on the basis of underlying diagnosis, treatment with TNF-inhibitors, or particle burden and size without sacrificing on statistical validity.

In addition, the immunohistochemistry performed in this study was mainly intended to corroborate the findings on light microscopy. As such the cell surface markers used were meant to detect T lymphocytes, B lymphocytes, and plasma cells. As discussed, however, other subsets of innate and adaptive immune system cells have been implicated in RA. These would include activated macrophages, mast cells, Th1 lymphocytes, and Th17 cells, which can be detected by EMR1-F4/80, CD117, CD26, and CD161 cell surface markers respectively[51-54]. Staining for these markers would provide a better sense of the absolute numbers of these cells present in the tissue reaction of RA patients, which could help further clarify the pathogenesis and natural history of RA in the context of wear debris.

Furthermore, one of the potential clinical implications of this study is the possibility of identifying new pharmacologic targets that could more effectively prevent aseptic loosening from peri-prosthetic osteolysis in RA patients. Future studies could examine the effectiveness of anti-plasma cell therapy. Such an approach might not only reduce the production of anti-CCP antibodies but also reduce the known inflammatory reaction induced by metallic wear debris. Medications such as bortezomib, which are
used in the context of multiple myeloma, already exist. Moreover, although TNF inhibitors appear to somewhat mitigate the inflammatory response seen in RA patients, osteolysis is not significantly affected. In order to more directly prevent osteolysis, pharmacologic targets could include the RANK/RANK ligand complex, IFN-γ, IL-17, or inducible nitric oxide synthase activation.

Finally, examining some of the clinical and biomechanical factors that affect the speed of implant failure would be another future direction. For instance, establishing the prevalence of overuse syndromes in causing device failure would provide anticipatory guidance in terms of counseling future arthroplasty patients with or without RA. Future studies could also probe changes to the devices themselves to reduce the baseline inflammatory response to wear debris. The lymphoplasmacytic response to foreign body wear debris suggests that adjusting material choice, density, and manufacturing properties could further mitigate the inflammatory response. Altering varus-valgus motion at the prosthetic joint or the range of motion, for example, could also be explored in order to decrease the speed of implant failure.
References


