Do Tissues From THA Revision of Highly Crosslinked UHMWPE Liners Contain Wear Debris and Associated Inflammation?

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Abstract

Background Polyethylene wear debris is a major contributor to inflammation and the development of implant loosening, a leading cause of THA revisions. To reduce wear debris, highly crosslinked ultrahigh-molecular-weight polyethylene (UHMWPE) was introduced to improve wear properties of bearing surfaces. As highly crosslinked UHMWPE revision tissues are only now becoming available, it is possible to examine the presence and association of wear debris with inflammation in early implant loosening.

Questions/purposes We asked: (1) Does the presence of UHMWPE wear debris in THA revision tissues correlate with innate and/or adaptive immune cell numbers? (2) Does the immune cell response differ between conventional and highly crosslinked UHMWPE cohorts?

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Methods We collected tissue samples from revision surgery of nine conventional and nine highly crosslinked UHMWPE liners. Polarized light microscopy was used to determine 0.5- to 2- μ m UHMWPE particle number/mm², and immunohistochemistry was performed to determine macrophage, T cell, and neutrophil number/mm².

Results For the conventional cohort, correlations were observed between wear debris and the magnitude of individual patient macrophage ($\rho = 0.70$) and T cell responses ($\rho = 0.71$) and between numbers of macrophages and T cells ($\rho = 0.77$) in periprosthetic tissues. In comparison, the highly crosslinked UHMWPE cohort showed a correlation between wear debris and the magnitude of macrophage responses ($\rho = 0.57$) and between macrophage and T cell numbers ($\rho = 0.68$). Although macrophages and T cells were present in both cohorts, the highly crosslinked UHMWPE cohort had lower numbers, which may be associated with shorter implantation times.

Conclusions The presence of wear debris and inflammation in highly crosslinked UHMWPE revision tissues may contribute to early implant loosening.

Introduction

The generation of polyethylene (UHMWPE) wear debris is a major cause of aseptic loosening, and the most common reason for THA revisions [63, 74]. The interaction of particles and cells in periprosthetic tissue and surrounding bone contributes to the deregulation of bone homeostasis resulting in osteolysis at the bone–implant interface [27, 61, 63]. Consequently, highly crosslinked UHMWPE (HXLPE) was introduced to improve wear properties of the bearing surface [8, 32, 44, 54, 57], and in vitro studies have shown 40% to 95% reductions in wear rate as compared

with conventional UHMWPE [37, 53, 58]. However, the brittle nature of the HXLPE [11], generation of predominantly submicron-sized particles [55, 61, 64], and studies showing the effect of particle size, shape, and number raise concerns regarding the long-term clinical performance of HXLPE [30, 43, 69, 73].

Based on previous immune cell studies and clinical experience with conventional UHMWPE, the initial inflammatory response to wear debris primarily involves monocytes/macrophages. These innate immune cells differentiate into histiocytes or fuse with other macrophages to form multinucleated giant cells [31, 33, 66]. The ingestion of wear debris by macrophages results in their activation, gene expression, and proinflammatory cytokine (eg, interleukin 1 β , tumor necrosis factor α , interleukin 6, prostaglandin E2, interleukin 8) and chemokine (eg, monocyte chemoattractant protein 1, macrophage inflammatory protein 1 α) secretion [1, 24, 26, 47, 61]. Collectively, these factors induce infiltration, maturation, and activation of immune cells and osteoclasts [35, 61, 62]. Another innate immune cell, the neutrophil, also ingests particles and releases proinflammatory factors; however, these cells are present only in low numbers in aseptic loosening [59].

The adaptive immune response includes several subgroups of T lymphocytes (T cells): T helper cells (T_H), involved in activating and directing other immune cells; cytotoxic T cells (T_C), which cause cell death in response to the recognition of a foreign or altered self-antigen; and regulatory T cells (T_{reg}), which suppress activation of the immune system maintaining homeostasis [18, 63]. Specifically, T_H cells play a major role in releasing cytokines (eg, RANKL) that promote macrophage differentiation into osteoclasts [10, 23]. Although the role of T cells in aseptic loosening is controversial, a recent study has identified a functionally active subset of T_C cells capable of downregulating T_H cells [65], which may explain the inconsistent detection of lymphokines in tissues around loosened prostheses [45, 65]. Additional studies showing correlations between increased numbers of T_H and T_C cells and radiographic osteolysis [28, 61] further implicate T cell involvement in bone remodeling. However, others only attribute a substantial involvement of T cells in the inflammatory Type IV hypersensitivity response to metal particles and/or ions [13, 48, 60, 76]. Thus, understanding of adaptive immune responses contributing to osteolysis lacks a clear consensus.

We recently reported the presence of histomorphologic changes in HXLPE revision tissues [8]. Specifically, our results showed a prevalence of fibrocartilage in tissues from HXLPE revisions implanted for less than 3 years (four of nine patients), implicating poor osseointegration in the development of loosening [71]. In the current study, we

sought to more closely investigate the role of UHMWPE wear debris in promoting inflammation and implant loosening by quantitatively measuring the in vivo inflammatory response.

Therefore, we asked: (1) Does the presence of UHMWPE wear debris in THA revision tissues correlate with innate and/or adaptive immune cell numbers? (2) Does the immune cell response differ between conventional and HXLPE cohorts?

Materials and Methods

We collected tissue samples from 18 consenting patients undergoing revision THA of uncemented, metal-on-UHMWPE hip components using a standardized tissue retrieval protocol; these are the same tissues used in the previous study [8]. UHMWPE liners were classified into two cohorts: conventional gamma air-sterilized UHMWPE (n = 9) and gamma inert-sterilized HXLPE (n = 9). For the conventional UHMWPE cohort, components were implanted for an average of 13.3 years (range, 9.6–15.6 years) and revised for femoral loosening (n = 3), acetabular loosening (n = 2), loosening of both components (n = 1), or wear debris and associated osteolysis (n = 2). For the HXLPE cohort, components were implanted for an average of 3.3 years (range, 1.5–5.9 years) and revised for femoral loosening (n = 4), acetabular loosening (n = 3), loosening of both components (n = 1), or subluxation (n = 1). Patients with known infection before surgery, implantation time less than 1 year, hepatitis, or cancer were excluded. Gender was represented equally in the two groups. We had prior IRB approval.

Tissues were excised from locations adjacent to the implant, which included the pseudocapsule for all 18 THA revision surgeries, and periprosthetic retroacetabular (acetabular loosening) or proximal femoral tissue (femoral loosening). The clinical details for the tissue samples are provided for the conventional (Table 1) and HXLPE (Table 2) materials.

To determine the quantity of UHMWPE wear debris, overlapping full-field tissue arrays were collected in polarized light using an Olympus BX50 microscope (Olympus Corp, Tokyo, Japan), equipped with a stepper motor-controlled stage ($\pm$ 2- μ m positioning accuracy) and elliptically polarized light imaging system (EPLIS) filters. EPLIS relied on the use of a $\frac{1}{4}$ wavelength birefringent quartz retardation plate, a calibrated rotating polarizing element, and a rotating interference filter, which were adjusted to achieve maximum particle/tissue contrast and to eliminate collagen and other nonparticle birefringence, to ensure only UHMWPE particles were observed. Initial validation of EPLIS was performed using scanning electron

Table 1. Clinical information, immune response, and particle number for conventional UHMWPE revision tissues

Patient	Implant time (years)	Gender	Revision reason	Tissue region	PMN (MPO)/mm ²	MAC (CD68)/mm ²	T cell (CD3)/mm ²	UHMWPE wear/mm ² (0.5–2 µm)	Cell total/mm ²
1	9.6	Female	Wear, osteolysis	C	5.6 ± 1.3	16.5 ± 5.1	0.6 ± 0.2	37.3 ± 11.7	629.7 ± 54.8
2	11	Male	Femoral loosening	C	0.0 ± 0.0	0.3 ± 0.1	0.0 ± 0.0	3.8 ± 0.8	540.2 ± 14.8
				PF	0.3 ± 0.2	559.3 ± 39.4	28.3 ± 4.6	48.8 ± 10.8	1167.0 ± 106.3
3	12.7	Female	Acetabular loosening	C	0.2 ± 0.1	186.2 ± 25.2	6.4 ± 1.2	14.6 ± 3.0	1053.0 ± 45.4
				RA	0.0 ± 0.0	494.8 ± 55.4	53.0 ± 8.5	151.9 ± 32.0	1139.0 ± 47.8
4	13.1	Male	Wear, osteolysis, femoral loosening	C	0.0 ± 0.0	5.6 ± 1.2	6.0 ± 1.8	3.0 ± 1.30	1006.3 ± 173.6
				PF	0.1 ± 0.1	491.8 ± 91.9	39.5 ± 9.9	5.5 ± 1.2	1405.6 ± 85.6
5	13.4	Female	Femoral loosening, wear	C	0.1 ± 0.1	3.4 ± 0.9	0.0 ± 0.0	1.6 ± 0.4	756.3 ± 47.2
6	14.2	Female	Femoral loosening	C	3.2 ± 1.5	342.0 ± 60.4	0.0 ± 0.0	5.7 ± 2.0	934.3 ± 103.7
				PF	1.5 ± 0.4	490.5 ± 44.5	9.1 ± 2.2	4.5 ± 0.8	839.3 ± 157.6
7	15.1	Male	Wear, osteolysis	C	0.4 ± 0.2	8.6 ± 2.4	0.8 ± 0.6	1.9 ± 0.5	619.7 ± 21.7
8	15.4	Male	Wear, osteolysis, acetabular and femoral loosening	C	1.5 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	2.1 ± 0.4	231.3 ± 39.2
				RA	8.4 ± 1.3	404.5 ± 23.2	33.6 ± 4.0	116.3 ± 20.7	1046.3 ± 99.9
				PF	5.4 ± 1.2	475.0 ± 26.4	43.1 ± 7.3	546.4 ± 70.8	1242.3 ± 74.9
9	15.6	Female	Wear, osteolysis	C	1.4 ± 0.4	370.1 ± 32.8	59.9 ± 12.7	35.5 ± 5.9	1353.3 ± 135.4

Values are presented as the average number of immunohistochemically positive cells, total cells, and 0.5- to 2-µm UHMWPE particles/mm² ± standard error of the mean; PMN = neutrophil polymorphonuclear leukocytes; MPO = myeloperoxidase; MAC = macrophage; CD = cluster of differentiation; C = pseudocapsule; PF = proximal femur; RA = retroacetabulum.

Table 2. Clinical information, immune response, and particle number for highly crosslinked UHMWPE revision tissues

Patient	Implant time (years)	Gender	Revision reason	Tissue region	PMN (MPO)/mm ²	MAC (CD68)/mm ²	T cell (CD3)/mm ²	UHMWPE wear/mm ² (0.5–2 µm)	Cell total/mm ²
1	1.5	Female	Acetabular loosening	C	0.9 ± 0.3	1.4 ± 0.4	1.3 ± 0.4	0.4 ± 0.1	539.6 ± 43.2
2	1.6	Male	Femoral loosening	C	1.0 ± 0.1	1.7 ± 0.3	0.4 ± 0.2	0.5 ± 0.1	567.0 ± 13.5
				PF	0.0 ± 0.0	3.5 ± 0.8	0.0 ± 0.0	1.4 ± 0.7	735.3 ± 15.2
3	1.7	Female	Acetabular and femoral loosening	C	8.6 ± 2.2	0.6 ± 0.4	0.7 ± 0.4	1.1 ± 0.3	911.3 ± 72.9
4	2	Male	Femoral loosening	C	0.4 ± 0.2	18.3 ± 8.1	3.3 ± 1.3	1.0 ± 0.2	854.3 ± 68.9
				PF	0.5 ± 0.2	69.6 ± 10.1	4.4 ± 0.9	0.2 ± 0.1	975.3 ± 43.9
5	2.7	Female	Acetabular loosening	C	0.5 ± 0.2	15.0 ± 1.8	16.7 ± 3.4	1.0 ± 0.2	945.3 ± 72.6
6	3.7	Female	Femoral loosening	C	0.2 ± 0.1	0.8 ± 0.3	0.9 ± 0.4	3.3 ± 0.6	609.6 ± 53.4
				PF	3.4 ± 1.0	108.3 ± 29.6	14.0 ± 3.5	8.8 ± 1.7	1196.7 ± 137.5
7	5.2	Female	Acetabular loosening	C	4.9 ± 1.1	50.9 ± 9.8	5.9 ± 1.2	4.7 ± 0.6	1304.6 ± 45.5
				RA	2.9 ± 0.8	2.0 ± 0.8	2.4 ± 0.6	2.9 ± 0.6	1600.7 ± 86.5
8	5.2	Female	Subluxation	C	3.0 ± 0.9	1.5 ± 0.4	0.4 ± 0.2	0.5 ± 0.2	757.3 ± 49.4
9	5.9	Male	Femoral loosening	C	0.5 ± 0.3	2.1 ± 0.3	2.3 ± 1.1	0.9 ± 0.3	666.3 ± 16.5

Values are presented as the average number of immunohistochemically positive cells, total cells, and 0.5- to 2-µm UHMWPE particles/mm² ± standard error of the mean; PMN = neutrophil polymorphonuclear leukocytes; MPO = myeloperoxidase; MAC = macrophage; CD = cluster of differentiation; C = pseudocapsule; PF = proximal femur; RA = retroacetabulum.

microscopy in a prior study of histomorphologic changes and wear debris in periprosthetic hip tissues [8].

To evaluate the involvement of inflammatory cells in mediating implant failure, periprosthetic hip tissues were

subjected to immunohistochemistry. Three 6-µm serial sections from each of the 18 patients' tissues were mounted on Fisherbrand® Superfrost® Plus slides (ThermoFisher Scientific, Inc, Waltham, MA, USA), dewaxed, and

rehydrated. A Dako automated slide stainer (Dako, Carpinteria, CA, USA) was used for the immunohistochemical staining reactions. All antibodies were diluted in 0.1 mol/L Tris/2% fetal bovine serum. For neutrophil detection, myeloperoxidase (MPO) rabbit polyclonal antibody (Dako) was used at a 1:4000 dilution for 30 minutes at room temperature. Prior epitope retrieval was for 10 minutes in pepsin (Dako). Secondary biotinylated anti-rabbit immunoglobulin (Ig) G (Vector Laboratories, Inc, Burlingame, CA, USA) was used at 1:200 dilution for 30 minutes at room temperature. For macrophage detection, cluster of differentiation 68 (CD68) mouse monoclonal antibody (Dako) was used at a 1:2000 dilution for 30 minutes at room temperature. Prior epitope retrieval was for 10 minutes in pepsin. Secondary biotinylated anti-mouse IgG (Vector Laboratories) was used at 1:200 dilution for 30 minutes at room temperature. For T cell detection, CD3 rabbit polyclonal antibody (Dako) was used at a 1:100 dilution for 30 minutes at room temperature. Prior epitope retrieval was for 20 minutes in Invitrogen™ (Life Technologies Corp, Carlsbad, CA, USA) EDTA epitope retrieval solution. Secondary biotinylated anti-rabbit IgG was used at 1:200 dilution for 30 minutes at room temperature. After rinsing, the slides were incubated with avidin biotin complex (Vector Laboratories) for 30 minutes at room temperature, rinsed, and incubated with diaminobenzidine (Dako) for 10 minutes at room temperature. Slides were counterstained with Harris hematoxylin (ThermoFisher Scientific).

To determine the number of positive cells and total cells, overlapping full-field tissue arrays were collected, and an image analysis program was developed to threshold bright-field images based on predetermined criteria of cell size, nuclear color, and staining intensity using ImagePro® Plus (Media Cybernetics Inc, Bethesda, MD, USA). Total cell numbers were determined as a measure of tissue integrity and loose versus dense connective tissue. To calculate tissue area, we applied a separate threshold to distinguish between the tissue and the white background. A quantitative value of inflammatory response then was determined as the number of positive cells/mm² tissue area. Finally, polarized light images were analyzed to determine 0.5- to 2-μm UHMWPE particle number using a customized image threshold operation programmed in MATLAB® (The Mathworks Inc, Natick, MA, USA). All imaging analyses were performed by three observers (RMB, TAF, LLJ) using the same software to threshold images from two patients to determine user variability. The results agreed within 95% of each other.

For both cohorts, we determined the number of UHMWPE wear particles, macrophages, T cells, and neutrophils in each corresponding polarized light and bright-field image, and the data were expressed as number/mm².

Because the data were not normally distributed, nonparametric statistics were used throughout. We assessed correlations between the number of macrophages, T cells, neutrophils, and UHMWPE wear debris using the Spearman rank correlation method for individual images and for the magnitude of individual patient responses (combined image results for each patient). When comparing all four data sets, the Kruskal-Wallis test was significant for macrophages and T cells, but not for neutrophils. Strictly where indicated, differences in the median number of immune cells/mm² were compared by the Wilcoxon Mann-Whitney test for each UHMWPE cohort (conventional versus HXLPE) and tissue region (pseudocapsular versus periprosthetic). Based on a prior evaluation of the same patient tissues, the sample sizes in the current study were sufficiently powered (> 0.9) to identify similar differences in inflammatory cell responses [8]. Statistical analysis was performed using JMP 8.0 (SAS Institute Inc, Cary, NC, USA).

Results

For the conventional UHMWPE cohort, substantial inter-patient and inpatient variability was observed in the number and location of UHMWPE wear debris and immune cells (Table 1). In general, the pseudocapsular and periprosthetic tissues from this cohort contained predominantly macrophages (Fig. 1A), followed by T cells (Fig. 1B), and a few neutrophils (Fig. 1C) were observed in some tissues (< 6/mm²). For the HXLPE cohort, heterogeneous immune cell responses also were observed in pseudocapsular and periprosthetic tissues (Table 2). Similar to the conventional cohort, the inflammatory cells observed in tissues from this cohort were predominantly macrophages (Fig. 1D), then T cells (Fig. 1E), and only a few neutrophils were observed (< 8/mm²) (Fig. 1F).

Owing to variability, we observed no correlation between the number of UHMWPE particles and immune cells from individual images of pseudocapsular and periprosthetic tissues when all nine patients in the conventional cohort were grouped. However, when considering the magnitude of individual patient responses (the combined image results for each patient), strong correlations were observed between macrophages and associated UHMWPE wear debris ($\rho = 0.69$; $p = 0.0042$) (Fig. 2A) and between UHMWPE wear debris and T cells ($\rho = 0.71$; $p = 0.0032$) (Fig. 2B). In addition, the magnitude of the macrophage and T cell responses was highly correlated for each of the nine patients and combined regions ($\rho = 0.77$; $p = 0.0008$) (Fig. 2C). For the HXLPE cohort, comparing

Fig. 1A–F Representative images of immunohistochemistry for innate and adaptive immune cells in periprosthetic hip tissue are shown: **(A)** macrophages (CD68+, red arrow), **(B)** T cells (CD3+, blue arrow), and **(C)** neutrophils (MPO+, green arrow) in patient tissues from the conventional UHMWPE cohort; and **(D)** macrophages

(red arrow), **(E)** T cells (blue arrow), and **(F)** neutrophils (green arrow) in patient tissues from the HXLPE cohort (nuclear stain, Harris hematoxylin; main image magnification, $\times 100$; inset images, enlarged by 200%).

Fig. 2A–C UHMWPE wear debris is associated with immune cells in pseudocapsular and periprosthetic tissues from revised conventional UHMWPE liners. Correlations were observed between wear particle number and individual patient responses for

(A) CD68+ macrophages ($p = 0.69$; $p = 0.0042$) and **(B)** CD3+ T cells ($p = 0.71$; $p = 0.0032$) and **(C)** separately for macrophages and T cells ($p = 0.77$; $p = 0.0008$) using Spearman rank correlation tests. CPE = conventional UHMWPE.

trends in the magnitude of individual patient responses, a moderate correlation was observed between macrophages and UHMWPE wear debris ($p = 0.57$; $p = 0.0422$) (Fig. 3A). In addition, the magnitude of the macrophage and T cell responses was moderately correlated for each of the nine patients and combined regions ($p = 0.68$; $p = 0.0103$), although the numbers for both were low in five of the patient tissues (Fig. 3B).

For the conventional and HXLPE cohorts, variability in the immune cell responses in pseudocapsular and periprosthetic tissues was observed (Table 3). However, comparison of the two cohort responses showed the

average number of macrophages/ mm^2 in periprosthetic tissue from the conventional cohort was higher than pseudocapsular tissue ($p = 0.0027$) from the same cohort and pseudocapsular ($p = 0.0018$) and periprosthetic tissues ($p = 0.0142$) from the HXLPE cohort (Fig. 4). The average number of T cells/ mm^2 also was greater in periprosthetic tissues from the conventional cohort as compared with pseudocapsular tissue ($p = 0.0148$) from the same cohort and pseudocapsular ($p = 0.0027$) and periprosthetic tissues ($p = 0.0252$) from the HXLPE cohort. The number of neutrophils was low regardless of UHMWPE cohort or tissue region.

Fig. 3A–B UHMWPE wear debris is associated with immune cells in pseudocapsular and periprosthetic tissues from revised HXLPE liners. Correlations were observed between wear particle number and the individual patient response for (A) CD68+ macrophages ($\rho = 0.57$; $p = 0.0422$) and (B) separately for macrophages and CD3+ T cells ($\rho = 0.68$; $p = 0.0103$) using Spearman rank correlation tests.

Table 3. Summary of pseudocapsular and noncapsular tissue responses for conventional and highly crosslinked UHMWPE implant revisions

Tissue type	Conventional UHMWPE		Highly crosslinked UHMWPE	
	n/N	Number/mm ²	n/N	Number/mm ²
Pseudocapsular tissue				
Neutrophils	7/9	1.4 (0.0–5.8)	9/9	2.2 (0.3–8.6)
Macrophages	8/9	111.6 (0.0–403.3)	9/9	10.0 (0.6–46.7)
T cells	5/9	8.7 (0.0–64.4)*	9/9	3.6 (0.4–17.9)
0.5- to 2- μ m UHMWPE particles	9/9	10.7 (0.3–34.2)	9/9	2.3 (0.5–8.6)
Total cells		791.6 (231.3–1353.3)		795.1 (539.7–1304.7)
Noncapsular tissue				
Neutrophils	5/6	2.7 (0.0–8.4)	3/4	1.7 (0.0–3.2)
Macrophages	6/6	470.9 (401.1–581.6)	4/4	46.4 (2.1–118.6)
T cells	6/6	33.7 (9.6–51.6)	3/4	5.2 (0.0–13.7)
0.5- to 2- μ m UHMWPE particles	6/6	205.0 (4.0–642.5)	4/4	4.2 (0.4–13.3)
Total cells		1139.9 (839.3–1405.7)		1127.0 (735.3–1600.7)

Values are presented as the average number of immunohistochemically positive cells, total cells, and 0.5- to 2- μ m UHMWPE particles/mm², with range in parentheses; *the number of pseudocapsular tissues showing a T cell response was lower in the conventional UHMWPE cohort than the incidence of other immune cell responses; n = incidence, N = total number of tissues.

Discussion

Polyethylene wear debris is a major contributor to inflammation in periprosthetic tissues and the development of osteolysis and loosening at the bone–implant interface. To reduce wear debris generation, HXLPE was introduced to improve wear properties of the bearing surface. However, only as THA revision tissues from HXLPE become available can the potential role of wear debris and inflammation in implant loosening be investigated. Therefore, we asked: (1) Does the presence of UHMWPE wear debris in THA revision tissues correlate with innate and/or adaptive immune cell numbers? (2) Does the immune cell response differ between conventional and HXLPE cohorts?

We acknowledge limitations of our study. First, there is a difference between the two patient cohort implantation times. The average implantation time was 13.3 years

(range, 9.6–15.6) for the conventional cohort and 3.3 years (range, 1.5–5.9) for the HXLPE cohort. Although implantation duration may affect the amount of wear debris and longevity of the immune response, the parameter we are reporting is local cellular response to existing wear particle load. Therefore, despite a reduced implantation time for the HXLPE cohort, the magnitude of the macrophage response was correlated with wear debris accumulation in pseudocapsular and periprosthetic tissues. Second, we established a lower limit for detection of wear debris size (0.5 μ m). Hip simulator studies of wear debris observed by environmental scanning electron microscopy suggest the mean particle size range is 0.2 to 0.5 μ m for HXLPE liners [67, 77] and 0.3 to 0.8 μ m for conventional liners [16, 78]. Thus, there is a greater potential to underestimate particle number in the HXLPE cohort, which may account for the reduced correlation for

Fig. 4 Regional differences and inter-patient variability are present in the immune cell responses of revision tissues from conventional and HXLPE cohorts. Boxplots show the average number of immunohistochemically positive cells/mm². The number of CD68+ macrophages and CD3+ T cells was higher in noncapsular, periprosthetic tissues from the conventional UHMWPE cohort. The number of MPO+ neutrophils was consistently low for both cohorts. A secondary y-axis is provided for easier interpretation of the magnitude of T cell and neutrophil responses. Provided are medians with a boxed range of 25th to 75th percentiles and whiskers showing the 10th and 90th percentiles. Outliers are shown as open circles. C = pseudocapsule; NC = non-capsular periprosthetic; HX = highly crosslinked UHMWPE; CPE = conventional UHMWPE.

wear debris and inflammatory cells observed for this cohort. Third, the cohort sizes are small, but the differences observed in this and a prior study of the same patient tissues were supported by a power analysis indicating a power of greater than 0.90 [8].

Our findings for the conventional cohort are similar to those reported in other immunohistochemical studies of periprosthetic tissues from uncemented, gamma air-sterilized UHMWPE liners [3, 5, 12, 29, 40, 41]. In general, the colocalization of wear debris and macrophages/histiocytes is well-established [28, 41], and several studies have identified the presence of T cells in periprosthetic tissues, with the incidence ranging from categorically absent to 30% [28, 40, 41]. However, in these studies, there was no attempt to correlate the presence of wear debris with immune cells, to separately evaluate the presence of macrophages and histiocytes using a size exclusion cutoff, or to determine macrophage and T cell number in the entire tissue section. Macrophages, unlike debris-laden histiocytes, have the potential to upregulate proinflammatory cytokine expression [49], and only by determining the number of these cells in each section can their involvement be assessed given the known intratissue variability [8, 28, 36, 40]. In our study [8] and others [28, 68], T cells were observed near blood vessels and in pseudocapsular and periprosthetic tissues associated with macrophages. Importantly, metal wear particles were not observed in patient tissues to account for the presence of T cells. Although animal models have shown T cells are dispensable in the development of osteolysis [25, 39, 72], a recent in vitro study showed depletion of T cells or the

addition of RANK-Fc to human peripheral blood cell cultures equally reduced osteoclast formation in response to RANKL [65]. Additionally, the current findings and those of others [28, 61] support the potential involvement of T cells in human osteolytic responses after recruitment by chemotactic factors released from activated macrophages [5, 19, 63, 68]. In agreement with other reports, we observed distinct regional and patient differences in the immune cell responses [8, 28, 36, 40] and notably a corresponding heterogeneity in UHMWPE particle number. The accumulation of UHMWPE wear debris in periprosthetic tissue may depend on several factors, other than their rate of production, including tissue morphology, particle size, and particle migration [6, 8, 15, 75]. In general, wear debris was observed predominantly in periprosthetic tissues removed from the bone-implant interface.

Similar to a previous study of two patient tissues from revised HXLPE liners, we found macrophages, a few lymphocytes, and limited amounts of UHMWPE wear debris [42]. However, immunohistochemistry was not done in that study [42] to quantify individual cell types or evaluate colocalization of the inflammatory response with wear debris. Using this approach, we found macrophage and T cell responses were moderately correlated for each of the nine patients and combined regions. The limited number of wear particles found in their study [42] and in ours may represent the greater potential for smaller particles to migrate away from the implant site. Alternatively, or in addition, nanometer-sized UHMWPE particles (< 0.5 μ m) may be present and undetected as even a small

wear volume would generate large numbers of predominantly submicron-sized particles [37, 38, 64].

The regional and patient differences in the number of macrophages and T cells are supported by previous investigations of immune cells and cytokines in tissues from loosened conventional prostheses [8, 29, 70]. In agreement with prior immunohistochemical [3, 5, 12, 28, 36, 40, 41] or histologic [42, 59] studies, the predominant cells in both cohorts were macrophages and T cells. The contribution of neutrophils was negligible, and even the largest neutrophil infiltration (8.6/mm²; Table 2) represented only a fraction of what would be considered evidence of subclinical infection [4, 7, 17, 56].

We performed a detailed assessment of UHMWPE wear debris (0.5–2 µm) and immune cells to compare the inflammatory response in revision tissues from conventional implants revised for osteolysis and loosening and HXLPE implants revised for loosening in the absence of radiographic osteolysis. Taken together, these results suggest the release of proinflammatory factors by activated macrophages may contribute to the recruitment of T cells, activation of osteoclasts, and osteolysis [2, 10, 34, 35, 61]. Alternatively, or in addition, it is possible submicron-sized particles induce poor osseointegration by directly inhibiting osteoblast viability and proliferation [14, 46]. Based on radiograph information alone, it is impossible to distinguish between implant loosening attributable to poor osseointegration and linear osteolysis. Furthermore, most cases of radiographic osteolysis are observed only after long-term (> 5 years) implantation as originally determined for conventional UHMWPE implants [9, 22, 53]. Nevertheless, the presence of wear debris and inflammation in HXLPE revision tissues, separately or in conjunction with osteoblast apoptosis, mechanical, and/or genetic factors [14, 20, 21, 46, 50–52, 71], may contribute to early implant loosening.

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