Proteins Bound to Polyethylene Components in Patients Who Have Aseptic Loosening After Total Joint Arthroplasty. A Preliminary Report

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Abstract

Background: Immunological responses to proteins that adhere to ultra-high molecular weight polyethylene have not, to our knowledge, been examined previously in patients who have aseptic loosening. In the current study, polyethylene components from forty-nine failed prostheses recovered during revision procedures were examined for the presence of antibodies that were bound to the polyethylene surface or that were reactive with other proteins that were bound to the polyethylene surface.

Methods: The polyethylene components consisted of thirty acetabular cups recovered during revision total hip arthroplasties and nineteen tibial components recovered during revision total knee arthroplasties. After extensive washing, bound proteins were extracted from the polyethylene components with use of 0.1-Molar glycine-hydrogen chloride solution followed by four-molar guanidine hydrochloride solution.

Results: Sufficient protein for analysis was recovered from forty-two polyethylene components. Polyacrylamide gel electrophoresis demonstrated a minimum of one and a maximum of twelve protein bands, with molecular weights ranging from thirteen to 231 kilodaltons. Immunoblotting revealed the presence of type-I collagen in most (thirty-four) of the forty-two explants, whereas aggrecan proteoglycans were detected in eight samples. Immunoglobulin also was detected in most (thirty-three) extracts, whereas type-II collagen was consistently absent. The presence of autologous antibodies directed against polyethylene-bound proteins in sera drawn at the time of the revision was investigated. Antibodies that were reactive against the ultra-high molecular weight polyethylene-bound proteins were detected in twenty-six of the forty-two patients with use of the Western blot technique. The number of reactive bands ranged from one to six, and the strongest binding was directed against a 103-kilodalton protein. Assays for specificity revealed that these sera autologous antibodies were reactive against the type-I collagen that was present in the explant solutions.

Conclusions: We hypothesize that immunoglobulin complexed with polyethylene may fix complement and that the complement cascade may in turn attract inflammatory cells to the polyethylene surface. Our data support the hypothesis that an immunological response to antigens bound to the polyethylene surface may contribute to aseptic loosening.

Clinical Relevance: Despite improvements in materials and designs of prostheses, aseptic loosening is the most common complication of total joint replacement, frequently leading to revision operations. We examined the immunological response to proteins that bind to ultra-high molecular weight polyethylene in patients who had aseptic loosening and discovered a high prevalence of antibodies to polyethylene-bound proteins. This immunological response may contribute to an inflammatory reaction in the periprosthetic tissue, ultimately leading to increased bone resorption around the prosthesis.

Osteolysis is a common complication of total joint replacement, and loss of support by the surrounding osseous architecture can lead to aseptic loosening of the prosthesis. The discovery of cytokines and the relationship between cellular signals and bone turnover has focused attention on biological processes that contribute to osteolysis11. However, the biological responses to plastics and metals are still poorly understood, and, to our knowledge, an immunological reaction directed against the implanted materials has not been seriously examined as a possible cause of prosthetic loosening.

Mechanical failure leads to the generation of small particles of biomaterials such as polymethylmethacrylate, ultra-high molecular weight polyethylene, and metal. Certain implanted polymers may cause immune reactions in a number of recipients15. It has been suggested that polymers may provide an adjuvant-like activity to native macromolecules, which adhere to

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hydrophobic surfaces and subsequently become immunogenic. Furthermore, ultra-high molecular weight polyethylene-bound proteins may represent antigens to preexisting antibodies in a number of patients who have a joint replacement, as autologous antibodies to connective-tissue antigens are common in patients who have osteoarthritis or rheumatoid arthritis.22-25.

The purpose of the current study was to investigate antibody reactions to proteins that adhere to ultra-high molecular weight polyethylene prosthesis components and to determine whether this polymer may provide a potential surface for the formation of immune complexes.

Materials and Methods

Patients

Patients who were scheduled to have a revision arthroplasty because of aseptic loosening, with the diagnosis based on the presence of osteolysis on roentgenograms, were selected for this study. All procedures for the study were approved by the Human Investigation Committee, and informed consent was obtained from all patients. Components of the failed prostheses and plain blood samples were obtained at the time of the revision operation.

Extraction of Proteins

Each failed ultra-high molecular weight polyethylene component was cleared of attached tissue by vigorous scrubbing with a nylon-bristle brush, and cement was removed mechanically. The component then was washed with use of end-over-end rotation in thirty milliliters of phosphate-buffered saline solution containing protease inhibitors, at 4 degrees Celsius, for thirty-four weeks with daily changes of the buffer. The entire component then was immersed in fifteen milliliters of 0.1-molar glycine-hydrogen chloride buffer (pH 2.8), vortexed for three minutes, and subjected to gentle agitation for an additional minute. The component was removed from the glycine-hydrogen chloride solution, which was adjusted to pH 7.5 by the addition of three-molar Tris buffer. It then was immersed in fifteen milliliters of four-molar guanidine hydrochloride solution, vortexed for three minutes, and subjected to gentle agitation for an additional minute. After removal of the component, this solution was adjusted to pH 7.5 by the addition of three-molar Tris buffer. After rapid dialysis against phosphate-buffered saline solution (pH 7.2), both solutions were concentrated with use of membrane ultrafiltration, and the protein content was determined with use of spectroscopic analysis at 280 nanometers and was adjusted to two milligrams per milliliter.

Analysis of Proteins with the Dot Blot Technique

The presence of connective-tissue antigens and immunoglobulin in the protein extracts was examined with use of the dot blot technique. Twenty microliters of saline solution containing five micrograms of protein extract was pipetted onto nitrocellulose paper strips and allowed to dry. The strips were blocked with 5 percent milk-phosphate-buffered saline solution at room temperature for five minutes and were placed in five milliliters of saline solution containing protease inhibitors, at 4 degrees Celsius, for thirty-four weeks with daily changes of the buffer. The strips were blocked with 5 percent milk-phosphate-buffered saline solution at room temperature for five minutes and were placed in five milliliters of saline solution containing protease inhibitors, at 4 degrees Celsius, for thirty-four weeks with daily changes of the buffer. The strips were washed with use of end-over-end rotation in thirty milliliters of saline solution or in serum-free 5 percent milk-phosphate-buffered saline solution for five minutes at four milliliters per track and once with phosphate-buffered saline solution for five minutes at five milliliters per track.

The strips were probed with use of goat anti-mouse antibody solution (3.0 milliliters of phosphate-buffered saline solution, 1.0 gram of milk, 1.0 milliliter of normal goat serum, and 0.06 milliliter of goat anti-mouse IgG conjugated to alkaline phosphatase [Fisher Scientific, Orangeburg, New York]) at room temperature for two hours. The control strips that had been placed in saline solution were reacted with adsorbed/clutched goat anti-human immunoglobulin (all isotypes) conjugated to alkaline phosphatase (Fisher Scientific) to assay for human immunoglobulins in the extracts. The strips were washed three times with 0.5 percent Tween-80-phosphate-buffered saline solution for five minutes at five milliliters per track and once with phosphate-buffered saline solution for five minutes at five milliliters per track. They then were placed in the aqueous substrate solution (0.2 milligram of nitro-blue tetrazolium per milliliter; 100-millimolar Tris-hydrogen chloride, pH 9.7; 0.5-millimolar MgCl2; 0.001-millimolar ZnCl2; 0.2 milligram of 5-bromo-4-chloro-3-indolyl phosphate [US Biochemicals, Cleveland, Ohio]) per milliliter, and, for 1.2 percent volume per volume dimethyl sulfoxide) for 2.5 minutes to develop. The development was stopped with water for twenty minutes at ten milliliters per track, and the strip was air-dried on paraffin.

The dot blots were scanned and analyzed with use of a Hewlett-Packard scanner (Mountain View, California) and with the Sigma-Scan image-analysis software package (Jandel Scientific, San Rafael, California).

Protein Electrophoresis

Proteins recovered from the polyethylene components were analyzed with use of sodium dodecyl sulfate polyacrylamide gel electrophoresis. The gel, containing the separated proteins or protein molecular weight standards, was fixed overnight in 250 milliliters of deionized water, fifty milliliters of 0.1-molar acetic acid, and 200 milliliters of methanol and then was silver-stained. The number and molecular weight of the protein bands were determined with use of a gel image-analysis scanning apparatus (Alpha Innotech, San Leandro, California).

Western Blot Technique

After completion of the electrophoretic separation, the electrophoresis gel was electrotransferred onto nitrocellulose paper with use of methods described previously. The gel, containing the separated proteins or protein molecular weight standards, was fixed overnight in 250 milliliters of deionized water, fifty milliliters of 0.1-molar acetic acid, and 200 milliliters of methanol and then was silver-stained. The number and molecular weight of the protein bands were determined with use of a gel image-analysis scanning apparatus (Alpha Innotech, San Leandro, California).

Results

Recovery of Proteins from Failed Polyethylene Components

Polyethylene components from forty-nine failed total joint prostheses were recovered during revision operations. Thirty acetabular components from total hip replacements and nineteen tibial components from total knee replacements were obtained (Table I). The prostheses were recovered after they had been in place for a minimum of nine months, with the exception of two

VOL. 81-A, NO. 5, MAY 1999
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**FIG. 1**

Number and molecular weight of protein bands in the polyethylene extracts, determined according to polyacrylamide gel electrophoresis.
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**FIG. 2**

Number and molecular weight of protein bands that were reactive with autologous sera drawn at the time of the revision operation, determined according to Western blot analysis. PAGE = polyacrylamide gel electrophoresis.
acetabular components (Implants 1 and 2, Table I) that had been removed during the primary operative procedure because of technical reasons. No proteins were recovered from these devices, and thus they were used as controls for the extraction procedure. A mean (and standard error) of 415 ± 59 micrograms (range, 4.70 to 2361 micrograms) of protein was recovered from the entire surface of the components. Forty-two extracts, which contained more than thirty-five micrograms of protein, were analyzed further with use of immunoblotting and electrophoresis.

**Analysis of Proteins with Use of Immunoblotting**

Forty-two protein extracts were analyzed with use of the immunoblot technique, which involved use of mouse antibodies specific for type-I and type-II collagen, aggrecan proteoglycans, and immunoglobulins (all iso-types). The immunoblots were classified as no antibody-binding (–); clear, positive antibody-binding within the blot area (+); or saturation antibody-binding within the blot area (++) (Table I).

Type-I collagen was detected in thirty-four of the extracts, and aggrecan proteoglycans were detected in eight. Type-II collagen was absent in all extracts. Immunoglobulin was detected in thirty-three extracts. The immunoglobulin was concentrated in the glycine-hydrogen chloride extraction solution, whereas the connective-tissue antigens were readily detected in both extraction solutions. The level of protein that was recovered from the components corresponded with the ability to detect type-I collagen; collagen was found in all explant solutions containing more than eighty-five micrograms of protein. In most instances, this relationship was also applicable to the ability to detect immunoglobulins, but this class of proteins was absent from two samples containing more than 100 micrograms of protein.

**Analysis of Protein with Use of Electrophoresis**

Forty-two guanidine hydrochloride extracts were analyzed for protein content with use of polyacrylamide gel electrophoresis and gel-staining. Positive bands were detected in thirty-four of the extracts, which reflects the limits of sensitivity of this analytical technique. In the thirty-four extract samples with a positive result on polyacrylamide gel electrophoresis, a mean (and standard deviation) of 5.6 ± 3.06 bands were detected, with a minimum of one protein band and a maximum of twelve protein bands. Twenty-one different proteins were identified in the extracts, and they ranged in molecular weight from thirteen to 231 kilodaltons (Fig. 1). The most frequently occurring proteins had molecular weights of 103 and sixty-seven kilodaltons (twenty-two and twenty-one samples, respectively).

**Antibody Responses to Polyethylene-Bound Proteins**

The Western blot technique was used to assess the presence of autologous antibodies against polyethylene-bound proteins. Forty-two extracts were electrotransferred to nitrocellulose, and the resulting strips were reacted with autologous sera and were probed with goat anti-human immunoglobulin. Because many of the extracts were positive for immunoglobulin on dot-blotting, control strips reacted with goat anti-human immunoglobulin (secondary antibody) alone were also included in the assay. Bands that were identified on both the control strips and the autologous antibody strips were disregarded in this assessment.

In one illustrative case (Fig. 3), the protein bands that were detected with use of silver-staining (lane 1) resulted in three immunoreactive bands after electrotransfer and Western blotting with use of autologous serum (lane 2). Autologous serum also generated a band at 103 kilodaltons with use of Western blotting against purified bovine type-I collagen (lane 3), which was coincidental with the band resulting from specific mouse anti-type-I collagen antibodies reacted against purified bovine type-I collagen (lane 4). Standard molecular weight (M.W.) markers also were electrophoresed (lane 5).

![Fig. 3](image-url)
# Levels of Proteins Extracted from the Entire Surface of Failed Ultra-High Molecular Weight Polyethylene Components, and the Presence of Connective-Tissue Proteins in the Protein Extracts

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*The immunoblots were classified as no antibody-binding (−), clear, positive antibody-binding within the blot area (+), or saturation antibody-binding within the blot area (++). NA = not available (immunoblotting was not done for these implants).
 ultra-high molecular weight polyethylene component.

Serum antibodies that were reactive with specific autologous polyethylene-bound proteins were detected in twenty-six of the forty-two patients. Fifteen different proteins generated specific positive reactions on the strips; the molecular weight of these proteins ranged from thirteen to 231 kilodaltons (Fig. 2). The most frequently immunoreactive proteins had molecular weights of 103 and 135 kilodaltons (twelve and ten samples, respectively).

**Antibodies Specific for Type-I Collagen**

Sera also were evaluated for reactivity with type-I collagen with use of the Western blot technique. All patients who were found to have an immunoreactive extract protein at 103 kilodaltons on assay with use of autologous sera also were found to have sera antibodies that were reactive against bovine type-I collagen on Western blots. Analysis of the sera from these patients revealed a single or duplex band that appeared to correspond with the 103-kilodalton molecular weight marker. This band position was identical to that of the strips that were developed with specific mouse anti-type-I collagen antiserum and were probed with goat anti-mouse antibodies. The control reactions of sera reacted against strips without collagen and the type-I collagen reacted against secondary antibody (goat anti-human IgG conjugated to alkaline phosphatase) alone were negative in this assay.

**Discussion**

Proteins absorbed to biomaterials have been implicated in the inflammatory response to polymers, possibly involving the activation of macrophages\(^3\). Our data demonstrate that a variety of proteins are strongly bound to the polyethylene components of prostheses; more than twenty different protein bands were detected in extracts analyzed with polyacrylamide gel electrophoresis. Type-I collagen was readily detected in most extracts with use of the immunoblot technique, and a band at 103 kilodaltons (presumably type-I collagen) was observed in many of the extracts. Our findings also suggest that most patients express antibodies that are reactive with the proteins bound to polyethylene and that type-I collagen is a major antigenic target in these patients. Several other groups of investigators have found antibodies to type-I collagen (particularly denatured collagen) in patients who have osteoarthritis\(^4,5\), although the prevalence and titer of these antibodies have been invariably lower than those of the antibodies to type-II collagen in patients who have rheumatoid arthritis\(^6\) and the immune response to connective-tissue antigens has not been implicated in the pathogenesis of degenerative joint disease. Jasim reported that cartilage samples from patients who had osteoarthritis contained sequestered immune complexes with antibodies to type-I collagen, a finding suggesting that a response to connective-tissue antigens is not uncommon in patients who have a joint replacement. Antibody-binding to denatured collagen was common in a study of a large orthopaedic population\(^7\), although operative procedures do not appear to increase the titers of anti-collagen antibodies to any major degree\(^8\).

The titers of antibodies to type-I collagen increase with age\(^9\), and positive titers have been observed in two (4 percent) of fifty normal blood donors\(^10\). However, the prevalence of antibodies to collagen in patients who have a revision operation is high, and it is possible that the implantation of a biomaterial, followed by the deposition of collagen, may contribute to increased levels of antibodies. This hypothesis has been proposed to account for the development of antibodies to an unusual collagen epitope in recipients of silicone implants\(^11\).

We hypothesize that immunoglobulin complexed with polyethylene may fix complement and that the complement cascade may in turn attract inflammatory cells to the polyethylene surface. Immunoglobulin bound to biomaterials has previously been implicated in inflammatory responses, as IgG-coated polymer implants were shown to activate human neutrophils in vitro and to attract murine phagocytes when they were implanted in vivo\(^12\). Since small ultra-high molecular weight polyethylene particles inevitably result from motion and wear of an orthopaedic prosthesis, the presence of polyethylene-bound proteins may be important when the particulate debris is engulfed by phagocytes in the tissue adjoining the site of the prosthesis. The phagocytosis of these particles may result in activated cells that secrete both proinflammatory cytokines and proteolytic enzymes and also provide activation signals to lymphocytes. It was demonstrated previously that mononuclear cells respond in vitro to polymethylmethacrylate in culture both by the secretion of the cytokines interleukin-1β and interleukin-2 and by cellular proliferation\(^13\). Therefore, particles of polyethylene could provide an adjuvant effect for the development of a response to polyethylene-bound proteins. Chronic, immunologically mediated inflammation may result from the reaction to prosthetic debris and associated immune complexes, thus damaging the tissue in the region of the prosthesis. It also has been suggested that the localized tissue response to implanted material may have an adverse effect on the chemical integrity of the polymer\(^14\). Our data thus support the hypothesis that an immunological response to polyethylene-bound proteins may contribute to aseptic loosening.

**References**


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