T-lymphocytes are not necessary for particulate polyethylene-induced macrophage recruitment

Histologic studies of the rat tibia

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Immunological processes involving T-lymphocytes have been implicated in the mechanisms of aseptic loosening of joint endoprostheses. We report the histological reaction of bone to phagocytosable particles of high-density polyethylene (HDPE) in normal and T-cell deficient rats. A bolus of 3×10^7 polyethylene particles averaging 4.7 μm in size, mixed in 0.1 mL of sodium hyaluronate, was injected into the right proximal tibia of 10 normal and 10 T-cell deficient (nude) Rowett rats from the same litter. The left control side was injected with sodium hyaluronate alone. The animals were killed after 6 weeks. Transverse paraffin-embedded sections stained with hematoxylin and eosin were made of the implant area.

On the control side, there was normal bone marrow without evidence of foreign body reaction. On the HDPE side, in both normal and T-cell deficient rats, macrophages were found to surround and engulf the particles, with no differences in the histological reactions. We conclude that T-lymphocytes are not necessary for the recruitment of macrophages to sites in which phagocytosable particles of HDPE have been implanted.

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The tissue surrounding loose cemented and cementless arthroplasty components is a pseudomembrane containing macrophages, foreign body giant cells and lymphocytes in a fibrous stroma (Bullough 1973, Willert and Semlitsch 1977, Mirra et al. 1982, Goldring et al. 1983). Particulate polymeric and metallic debris are also frequent findings. Whether immunological processes participate in this response is controversial. Santavirta et al. (1991, 1992) provided no histological evidence of activation of the immune system when examining the tissue interface surrounding revised uncemented acetabular cups. However, Lalor et al. (1990, 1991, 1992) implicated the presence of a Type IV, delayed hypersensitivity reaction to titanium debris in the tissues surrounding failed titanium alloy components in humans. Goodman et al. (1992) noted the presence of both macrophages and T-lymphocytes at the interface between cemented and cementless, loose and well-fixed hip and knee components undergoing revision. Furthermore, these cells expressed the mRNA sequences for cytokines and growth factors that can modulate the growth, proliferation, and differentiation of cells in the immune system and the monocyte/macrophage system, as well as mediate the remodeling of bone and other mesenchymal tissues (Huie et al. 1993).

Particles of polyethylene, similar to wear debris produced from arthroplasty components, have been shown to produce a foreign body and chronic inflammatory response when implanted in animals (Goodman et al. 1988, 1990, Howie et al. 1988). Whether T-lymphocytes are necessary for the recruitment of foreign body cells to the site of implantation of polyethylene debris is unknown. In this study we attempt to answer this question by implanting small phagocytosable polyethylene particles in normal and T-cell deficient rats.

Material and methods

Polyethylene particles

The generation of particles of ultra-high molecular weight polyethylene (UHMWPE), the material currently used for endoprostheses, is difficult. Recently this was accomplished by using an attrition technique, yielding fractions of different densities and with crystalline and amorphous phases (Leigh et al 1992).

Figure 1. Normal rat specimen. The medullary cavity contains marrow cells and bone. The positively birefringent polyethylene particles are surrounded and engulfed by macrophages. HE, ×200.

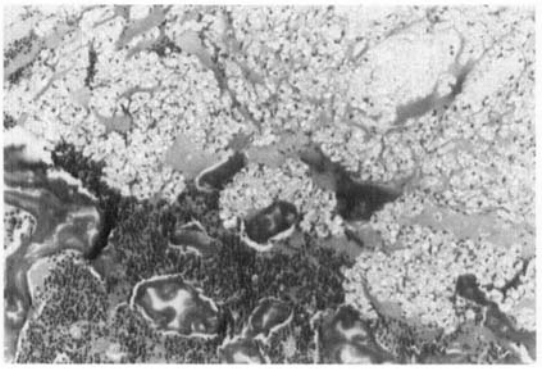

Figure 2. T-cell deficient (nude) rat specimen. Note that macrophages are seen in a relation to the positively birefringent polyethylene particles similar to that in the normal rats. HE, ×200.

These UHMWPE particles have not yet been fully characterized, and are not for general distribution to orthopedic researchers. We obtained small phagocytosable particles of high-density polyethylene (HDPE) (Shamrock Technologies, Newark, NJ, U.S.A.); this material is similar to UHMWPE, but is slightly more dense and has a lower molecular weight. The particles were produced by a proprietary technique, and were reported to be 100 percent pure and highly crystalline with a specific gravity of 0.95. The particles were globular shaped and averaged 4.7 ± 2.1 μm (mean ± standard deviation), using scanning electron microscopy.

Surgery

10 normal rats and 10 T-cell deficient (nude) Rowett rats, 6 weeks of age were used in the study. Each group of rats was bred at the Wallenberg Laboratories in Lund and derived from the same litters. After induction of general anesthesia, both limbs were shaved and painted with a chlorhexidene solution. All the animals underwent the same procedure. A 0.6-mm needle and a 1-mL syringe were used to aspirate approximately 0.1 mL of marrow contents by percutaneously penetrating the medial cortex of the proximal tibia immediately below the growth plate. Thereafter, 0.1 mL of 1% sodium hyaluronate (Healon, Kabi Pharmacia, Uppsala, Sweden) was injected into the left control tibia and on the contralateral right side, 0.1 mL of Healon containing 3×10^7 particles of HDPE were injected. 6 weeks later, the rats were killed by a barbiturate overdose. The proximal tibias were isolated, decalcified in Parengy's solution, embedded in paraffin, and multiple, 6 μm, transverse sections were cut and stained with hematoxylin and eosin. These sections were examined with a light microscope, using both transmitted and polarized light.

3 of the normal rats died immediately post-operatively due to an anesthetic overdose. 2 of the T-cell deficient rats died 4 weeks postoperatively. 2 of the normal rats were therefore killed at this time for comparison. The remaining animals survived without complications.

Results

The tibias from the rats that died immediately postoperatively demonstrated localized implantation sites in the proximal tibia, with minimal leakage in the surrounding tissues. The control side contained localized areas that stained light pink and were devoid of cells, consistent with sodium hyaluronate, intermingled with normal appearing marrow cells. The right side contained positively birefringent HDPE particles intermingled with normal marrow cells. No acute or chronic inflammatory or foreign body reactions were identified.

After 4 and 6 weeks, the control tibias contained normal marrow cells with a few small localized zones of fibrinous-like, pink staining material. These areas were surrounded by normal marrow cells. HDPE-implanted tibias contained positively birefringent particles surrounded and engulfed by numerous macrophages (Figure 1). Normal marrow constituents were contained in the surrounding bone cavity.

The T-cell deficient rat tibias implanted with Healon or HDPE particles were indistinguishable from their counterparts in the normal rats (Figures 2 and 3). Lymphocytes were present in the surrounding marrow. Specifically, the macrophage reaction seen in areas containing HDPE particles in the nude rats was similar to the reaction seen in the normal rats.

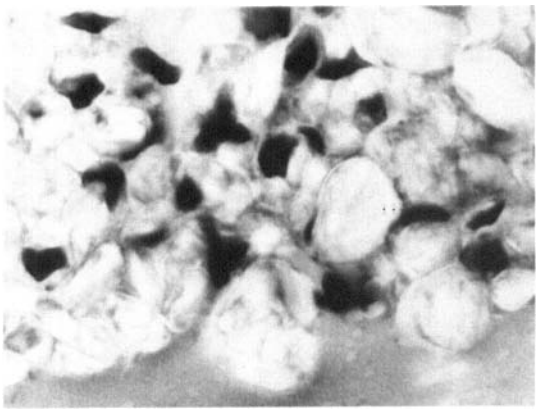

Figure 3. T-cell deficient (nude) rat specimen. The cytoplasm of macrophages surrounds the positively birefringent polyethylene particles, which indent and deform the nuclei. HE, ×1000.

Discussion

This study has shown that T-lymphocytes are not necessary for the recruitment and accumulation of macrophages and foreign body giant cells and subsequent phagocytosis of HDPE particles in the rat. Jeranik et al. (1993) have recently provided evidence that T- and B-lymphocytes as well as NK cells (activated killer cells) are not necessary for macrophage accumulation to an area implanted with polymethylmethacrylate particles. Our study was performed before their study was reported and confirms that macrophages are not dependent on T-lymphocytes for their initial recruitment to the implantation of particles of HDPE.

Are T-lymphocytes important to the process of aseptic loosening? Previous studies by our group using immunohistochemistry and in situ hybridization have noted greater numbers of total T-lymphocytes, cytotoxic/suppressor T-lymphocytes and helper T-lymphocytes, as well as macrophages surrounding loose, cemented arthroplasties with osteolysis, than from those without osteolysis (Huie et al. 1993). Furthermore, the levels of IL-6, TGFb, TNF, and PDGF were greater in the membranes from the former compared to the latter group. It is well established that lymphokines play a critical role in modulating the release of inflammatory mediators from macrophages that are associated with the resorption of bone (Unanue and Allen 1987, Johnston 1988, Renz et al. 1989). In this respect, other factors may be important, including patient characteristics, the nature of the materials used in the arthroplasty, the exposure time to these materials, and whether interfacial motion was present. Studies by Lalor et al. (1990, 1991, 1992) have suggested that at least some of these factors are important when considering orthopedic implants.

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References

Bullogh P G. Tissue reaction to wear debris generated from total hip replacement. The Hip Soc Proc. C V Mosby, St Louis 1973: 80–91.

Goldring S R, Schiller A L, Roelke M, Rourke C M, O'Neil D A, Harris W H. The synovial-like membrane at the bone-cement interface in loose total hip replacements and its proposed role in bone lysis. J Bone Joint Surg (Am) 1983; 65 (5): 575–84.

Goodman S B, Fornasier V L, Kei J. The effects of bulk versus particulate ultra-high-molecular-weight polyethylene on bone. J Arthroplasty (Suppl) 1988; 3: S41–6.

Goodman S B, Fornasier V L, Lee J, Kei J. The histological effects of the implantation of different sizes of polyethylene particles in the rabbit tibia. J Biomed Mater Res 1990; 24 (4): 517–24.

Goodman S B, Alpert S, Griffiths G, Issaz S, Lee K, Woolson S T, Schurman D J, Maloney W J, Weissman I. Immunohistochemical analysis of the membrane surrounding total joint arthroplasties. Trans Fourth World Biomaterials Conf 1992: 439.

Howie D W, Vernon Roberts B, Oakeshott R, Manthey B. A rat model of resorption of bone at the cement-bone interface in the presence of polyethylene wear particles. J Bone Joint Surg (Am) 1988; 70 (2): 257–63.

Huie P, Doshi A, Lee K, Woolson S T, Maloney W J, Schurman D J, Goodman S B, Sibley R. The mechanism of cell recruitment and osteolysis in total joint arthroplasty: Analysis of the membranous interface using immunohistochemistry and in situ hybridization. Trans Orthop Res Soc 1993: 244.

Jeranik W A, Wolfe H, Bragdon C R, Goldberg M J. The role of the immune system in the foreign body response to particulate debris: Granuloma formation in immunodeficient mice. Trans Orthop Res Soc 1993: 272.

Johnston R B Jr. Current concepts: immunology. Monocytes and macrophages. N Engl J Med 1988; 318 (12): 747–52.

Lalor P A, Revell P A. T lymphocytes and titanium aluminum vanadium (TiAlV) alloy. Evidence for immunological events associated with debris deposition. Trans Fourth World Biomaterials Conf 1992: 438.

Lalor P A, Freeman M A R, Revell P A. Immunological studies of the bone-implant interface. Trans 16th Annual Meet Soc Biomaterials 1990; 13: 203.

Lalor P A, Revell P A, Gray A B, Wright S, Railton G T, Freeman M A. Sensitivity to titanium. A cause of implant failure? J Bone Joint Surg (Br) 1991; 73 (1): 25–8.

Leigh H D, Taylor P, Swaney P, Black J. Research and development report: Production of fine particulate ultra high molecular weight poly (ethylene) for biological response studies. J Appl Biomaterials 1992; 3, 77–80.

Mirra J M, Marder R A, Amstutz H C. The pathology of failed total joint arthroplasty. Clin Orthop 1982; 170: 175–83.

Renz H, Gentz U, Schmidt A, Dapper T, Nain M, Gemsa D. Activation of macrophages in an experimental rat model of arthritis induced by Erysipelothrix rhusiopathiae infection. Infect Immun 1989; 57 (10): 3172–80.

Santavirta S, Konttinen Y T, Hoikka V, Eskola A. Immunopathological response to loose cementless acetabular components. J Bone Joint Surg (Br) 1991; 73 (1): 38–42.

Santavirta S, Gristina A, Konttinen Y T. Cemented versus cementless hip arthroplasty. A review of prosthetic biocompatibility. Acta Orthop Scand 1992; 63 (2): 225–32.

Unanue E R, Allen P M. The basis for the immunoregulatory role of macrophages and other accessory cells. Science 1987; 236 (4801): 551–7.

Willert H G, Semlitsch M. Reactions of the articular capsule to wear products of artificial joint prostheses. J Biomed Mater Res 1977; 11 (2): 157–64.