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THE EFFECT OF PARTICLES, INCLUDING NANOPARTICLES, ON MACROPHAGES *IN VITRO* AND *IN VIVO*

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Abstract

Macrophages remove foreign material from the body and are recruited to sites were there are particles present. Multinucleate giant cells form by the fusion of macrophages. In the presence of particles, macrophages produce various chemical mediators, known as cytokines, as well as enzymes. Some of the cytokines are pro-inflammatory (for example, IL1ß, IL6 and TNFα) while others promote giant cell formation (GM-CSF, M-CSF, TGF). The presence of these cellular products can be shown by examining tissue sections with immunohistochemistry and by western blotting. The message (mRNA) for the synthesis of these molecules can be demonstrated by in situ hybridization and the polymerase chain reaction.

Macrophages process ingested material and present it as antigen to lymphocytes. Antigen-presenting macrophages play an important part in the initiation of metal sensitization. Surface receptors and their counterligands are expressed on macrophages and lymphocytes during antigen presentation.

The particles present in tissues around joint prostheses have been isolated and characterized. Over 95% of these are less than 1 micron (ECD) in size. Transmission electron microscopy has revealed nanoparticles of metal in the range 15-20 nm. Such particles are too small to be phagocytosed. Hydroxyapatite, diamond-like carbon (nano-diamond) and metal particles are being studied and results compared with those of particles in the micrometre range. There is a different response to different nanoparticles.

Key words: *Macrophage; lymphocyte; particles; cytokines; immunity; sensitization; nanodiamond.*
[Engineering of Biomaterials, 56-57,(2006),29-31]

Introduction

After the acute phase of inflammation, macrophages and multinucleate giant cells (MNGC) are responsible for removing foreign material, micro-organisms and dead tissue from the body. MNGC are formed by fusion of macrophages. Both of these cells are recruited to sites were there are particles, for example, wear debris around prosthetic joints. Macrophages are derived from circulating monocytes, migrating from vessels. In the presence of particles, macrophages and MNGC produce chemical mediators, known as cytokines. These can be demonstrated in situ and their production can be induced in cell culture when macrophages are incubated with particles.

It is clear from in vitro studies that macrophages are induced to produce these factors when they phagocytose (engulf) particles. Recognition of foreign material depends on a variety of mechanisms including surface receptors for the Fc component of immunoglobulin and for complement. The particles found adjacent to prosthetic joint components are derived mainly from the load-bearing surfaces and shed into the synovial fluid. It is likely that particles in the body become rapidly coated with proteins, including albumin, globulins, and complement components.

An important function of some macrophages is the processing of ingested material and its presentation as antigen to lymphocytes, the specific mediator cells in immune responses. Antigen-presenting macrophages are found in relation to particles in vivo, and these cells play an important part in the initiation of the process of sensitization to metal. Surface receptors and counterligands are expressed on macrophages and lymphocytes and can be detected in cell culture and in tissue sections. Macrophages and MNGC also contain nitric oxide synthase (iNOS) which gives rise to nitric oxide, a signalling molecule, and superoxide dismutase (SOD), involved in oxygen free radical production. Acid phosphatase (AcP) and non-specific esterase (NSE) play a part in the breakdown of ingested biological material, though there is no intracellular mechanism for intracellular breakdown of particles of man-made materials, such as metal or polyethylene.

The particles present in tissues around joint prostheses have been isolated and characterized. While there are occasional large shards visible by light microscopy, over 95% of particles are less than 1 micron (ECD) in size [1-3]. Transmission electron microscopy has also revealed the presence in some samples of nanoparticles of metal, in the range 15-20 nm. Such particles could not be taken into the cells by the usual phagocytosis process and might be pinocytosed. The study of the effects of nanoparticles on inflammatory cells is at a preliminary phase. Hydroxyapatite, diamond-like carbon (nanodiamond) and metal particles are being studied and results compared with those of particles in the micrometre range.

Materials and methods

Detailed materials and methods are not provided here in this short review. The effect of micro- and nanoparticles on macrophages has been studied in tissue retrieved from man after revision surgery for prosthesis loosening and from animals after experimental procedures. Cell culture studies use primary monocytes and lymphocytes derived from peripheral blood (PBM, PBL) as well as cell lines. Methods of investigation include immunocytochemistry (IHC) with monoclonal antibodies (mab), western blotting, in situ hibridisation (ISH), polymerase chain reaction (PCR) and reverse transcriptase PCR (RT-PCR) as well as ELISA, biochemical assays (eg citrulline- arginine for iNOS) and enzyme histochemistry (eg for AcP and NSE).

Results and discussion

Macrophages and MNGCs

Examination of tissue sections from patients undergoing

revision surgery for aseptic loosening, shows the presence of a cellular infiltrate composed of macrophages, MNGCs and lymphocytes [4-6], though there are also mast cells, fibroblasts and endothelial cells forming new blood vessels. This description will concern itself only with the phagocytic cells and their relationship to lymphocytes. The macrophages and MNGCs can be labelled with mab CD68. When activated, these cells express HLA-DR and integrins (CD11a,b,c) at their surface membrane [7-9]. Double-labelling fluorescence immunocytochemisty shows that these activated cells contain a number of cytokines. Such cytokine production can be demonstrated in functional studies of PBM and the human monocyte/ macrophage cell line U937, using IHC and western blotting to demonstrate each cytokine in tissue and cells, ELISA to show the levels of cytokine released into tissue culture fluid and PCR for the mRNA of each cytokine in cells either in culture or in tissue. The various cytokines known to be produced on microparticle phagocytosis in culture or in vivo are shown in FIGURE 1 [9-17]. Some of these promote the inflammatory process (IL1ß, IL6, TNFα), some are anti-inflammatory (IL10), yet others promote MNGC or ostoeoclast formation (GM-CSF,M-CSF,TGFα -directly; IL1, TNF -indirectly).

Macrophage-lymphocyte interactions

The presence of lymphocytes intermingled with the macrophages and MNGCs was reported over a decade ago [18]. Accumulating evidence points to an immunologically mediated process in the reaction to wear particles [18-21]. There is clinical evidence of metal sensitization in some cases, while others with aseptic loosening show features of a T cell mediated process on immunohistological examination and cell culture. An important function of phagocytic cells is presentation of antigen to lymphocytes. Markers of antigen-presenting cells have been demonstrated on interface macrophages in aseptic loosening (RFD1, CD80, CD86) with the counterligand (CD28) found on the accompanying T helper (CD4) lymphocytes [21-24]. Other ligands and counterligands (HLA-DR, TCR; CD40, CD40L; ICAM, LFA-1) known to be expressed in antigen-presentation are present on the macrophages and lymphocytes associated with particles in situ [25]. Furthermore, the interaction between macrophages phagocytosing particles and T lymphocytes has been modelled in cell culture experiments. T lymphocyte activation and proliferation requires the presence of IL2 or IL15. The latter is present abundantly in the particle-related macrophage infiltrate and its production can be stimulated on phagocytosis of particles in cultured U937 cells [7, 26, 27].

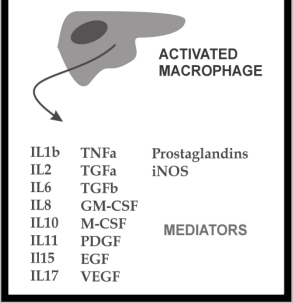

FIG.1. Cytokines and other mediators produced by actived macrophages.

Microparticles and nanoparticles

The studies described above refer to microparticles (>0.1µm diam). However, nanoparticles of titanium (15-20 nm diam) have been noted in cells from the implant interface by transmission electron microscopy in our own laboratory and by researchers in the USA. Little is known of how these nanoparticles enter the cell or of their effect on cell function, which has prompted our preliminary studies in this area. A recently reported experiment showed that nanoparticles of hydroxyapatite (nanoHA) increased the proliferation of Jurkatt T cells when cocultured with U937 cells. This effect was not due to IL15 production by U937 cells. More recent collaborative research has compared the effects of microparticles of CoCr alloy (1.3-2.4µm) with three different nanodiamond particles (0.15-0.81µm; 0.5-1.2µm aggregates; 0.2-0.6µm aggregates) on culture with U937 cells. The expression of HLA-DR, a sign of activation, and CD80, CD86 and CD40, indicators of antigen presentation, were measured using a FACS method. Positive control was provided by lipopolysaccharide (LPS) and negative control by culturing the cells in culture medium alone. CoCr, diamond particles (D1,DB) caused HLA-DR expression. CD86 was expressed with one of these diamond particles and CoCr. There was insignificant HLA-DR or CD86 expression the smallest diamond (D2). The results for CD40 are shown in FIGURE 2. All values are the means with standard errors (3 replicates for each macrophage stimulant). There was expression of CD40 by cells incubated with CoCr and the largest diamond particles (DB) after 24 hrs. This was respectively 3 and 4.5 times greater than CD40 expression in positive controls. There was much less CD40 expression in the case of the D1 and D2 nanodiamond particles. The results are normalized to the mean value for the negative controls, which contained no added particles or LPS.

Conclusions

Particles in the micrometre range (0.1-2.5µm) are phagocytosed by macrophages and cause activation of these cells on the evidence of tissue examination and cell culture studies. Some macrophages fuse to form giant cells (MNGC). Macrophages and MNGC produce numerous mediators and present antigen to lymphocytes upon activation. Particles in the nanometre range (<100nm) are also found in macrophages in tissue. Relatively little is known of their effects on cells but evidence is presented from preliminary studies that macrophage activation and antigen presentation is less with nanoparticles than that seen with microparticles.

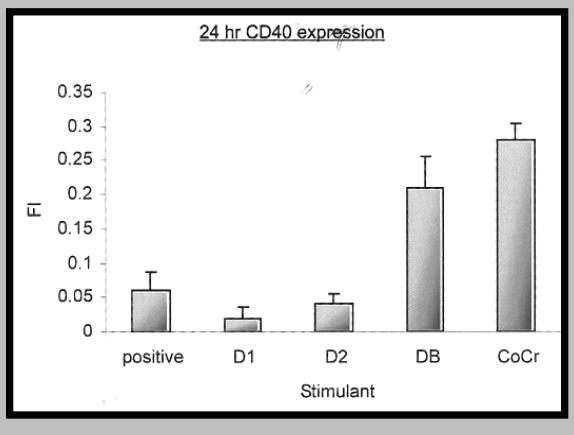

FIG. 2. Expression of CD 40 by U937 cells after incubation with different particles for 24 hrs. CoCr, cobalt chromium; DB, D1, D2, diamond; positive, LPS.

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THE INFLUENCE OF NANOCRYSTALLINE DIAMOND LAYERS OBTAINED BY MW/RF PECVD METHOD ON SURFACE PROPERTIES OF AISI 316 L STEEL

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Abstract

Determination of corrosion parameters of AISI 316 L with nanocrystalline diamond (NCD) layers deposited by means of new Microwave / Radio Frequency Plasma Enhanced Chemical Vapor Deposition (MW/RF PECVD) method in testing solution 0.5 M NaCl was a basic aim of presented work. We measured corrosion potentials, potentiodynamic characteristics, breakdown and repassivation potentials, corrosion resistance and impedance characteristics of AISI 316 L samples with- and without NCD layers. Summarizing obtained results it can be stated that NCD layers improve corrosion features of AISI 316 L and that surface preparation techniques have insignificant influence on these features.

Keywords: *biomaterials, austenitic steel AISI 316 L, NCD layers, electrochemistry, corrosion*
[Engineering of Biomaterials, 56-57,(2006),31-34]

Introduction

Since 1986 S. Mitura and co-workers from Technical University in Lodz have produced nanocrystalline diamond (NCD) layers on different substrates by means of Radio Frequency Plasma Enhanced Chemical Vapor Deposition Method (RF PECVD) [1-3]. In 2004 a new dual frequency - Microwave and Radio Frequency PECVD method was there applied for NCD layers deposition [4]. Dual frequency