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The role of newly formed vessels and cell adhesion molecules in the tissue response to wear products from orthopaedic implants

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Neovascularization and activation of endothelial cells play an important role in recruitment of blood leucocytes at sites of inflammation. This study aimed to assess the pattern of vascular growth and the expression of cell adhesion molecules on vascular endothelium and inflammatory macrophages and T cells in the bone–implant interface from patients with aseptically loosened orthopaedic prostheses. ELAM-1, VCAM-1, ICAM-1 and the receptors LFA-1 and CR3 were immunolocalized on cryostat sections of the interface obtained during revision arthroplasty. The results showed that ELAM-1 was restricted to endothelium and was upregulated on different vessels in 21 cases. Its expression correlated strongly with the presence of metal wear debris. VCAM-1 was less frequently expressed ($n=6$ cases), and was co-expressed with ELAM-1 in three cases with metal debris. ICAM-1 was detected on a large number of vessels on the bone side in 13 cases, but was more strongly expressed on macrophage subsets and foreign body giant cells (FBGCs) on the lining layer at the implant side. This study indicates the contribution of three different pathways in the migration of inflammatory cells to the bone–implant interface in response to phagocytosis of implant degradation products. Upregulated ELAM-1 expression may suggest a role in hypersensitivity reactions. Finally the persistent expression of VCAM-1 and ICAM-1 on macrophages and FBGCs in the lining layer indicates possible cellular interactions with the extracellular matrix proteins.

1. Introduction

The resorption of bone in the process of aseptic loosening of orthopaedic implants is well established, but recent evidence has shown that bone lysis may also occur in relation to stable, well-fixed devices [1, 2]. In both instances, the bone loss is linked to local inflammatory events in the bone implant interface [3]. A fibrous tissue layer containing few cells or vessels develops in relation to most non bioactive implants [4, 5], and such a fibrous reaction may be related to micromovement [6]. By contrast, the fibrous membrane recovered at the time of revision surgery for aseptic loosening exhibits high vascularity and a heavy cellular infiltrate, predominantly containing lymphocytes, macrophages and giant cells, recruited as a response to the presence of particulate wear debris [7–9]. The pathogenetic mechanisms whereby these cells gain access to the interface region are at present unclear. It is known from the general field of inflammation, tissue repair and the healing process that the formation of new vessels plays an important role in the delivery of cells to an inflammatory site [10]. The part which endothelial cells play in the recruitment of other cells and their migration into the tissues is becoming clearer, in that they play a key role in chemotaxis, cell adhesion and migration of leucocytes through vessel walls during inflammation [11, 12]. The adhesion molecules expressed by endothelial cells are induced by the local presence of cytokines [13–15]. The presence of such cytokines in the tissues adjacent to orthopaedic implants has been shown by ourselves and others [16, 17]. The adhesion molecules expressed by vascular cells are well characterized and can be localized in tissue sections by immunohistochemistry. Endothelial leucocyte adhesion molecule-1 (ELAM-1), also known as E-selectin, is a glycoprotein important for neutrophil recruitment as well as T lymphocyte and monocyte/macrophage adhesion [18–20]. ELAM-1 belongs to a family of adhesion molecules known as the LEC-CAMS [21, 22]. The other major adhesion molecules are vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule (ICAM-1) which are both members of the immunoglobulin supergene family [23]. VCAM-1 and ICAM-1 are both increased in expression (upregulated) on endothelial cells which are mediating the migration of leucocytes through their vessel walls. This migration is mediated by the binding of counter-receptors on the surface of leucocytes for these adhesion molecules, namely, VLA-4 recognized by VCAM-1 [24], LFA-1 and Mac-1 interactive with ICAM-1 [25, 26]. When inflammatory reactions take

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place in other sites, such as the synovium in arthritis, vascular growth and expression of these adhesion molecules on endothelial cells accompany the outpouring of cells belonging to the lymphocytes or macrophage populations [27]. With this in mind, we have performed a study of the possible part played by vascular endothelial adhesion molecules and their cellular ligands in the bone–implant interface, assessing their involvement in the cellular inflammatory reaction to particulate wear debris in aseptic loosening of orthopaedic devices.

2. Materials and methods

Fibrous tissue was studied from the bone–implant interfaces of 30 cases (Table I) where there was revision surgery being performed for osteolysis-associated aseptic loosening. There were 19 total hip and 11 total knee revisions which had been in place for 18 months to 22 years and 16 months to 15 years, respectively. Carefully orientated samples of fresh interface membrane measuring up to 4 mm in length were embedded in OCT, snap frozen in liquid-nitrogen-cooled isopentane before being stored at −70 °C. 5 μm cryostat sections were made from at least two representative samples from each case, air dried at room temperature for longer than 2 h, then briefly fixed in acetone/methanol (50/50) at −20 °C for 10 min and washed in 0.05 M tris HCl buffered saline (pH 7.6) (TBS). The panel of antibodies listed in Table II was used in a biotin streptavidin alkaline phosphatase method. Briefly, the tissue sections were incubated with these primary antibodies for 1 h at room temperature at the dilutions shown in Table II, followed by a 1 h incubation with biotinylated horse anti-mouse IgG, then alkaline phosphatase-streptavidin for a further hour, with 3 × 5 min washes in TBS between each of these stages. The exception to the above was the use of an indirect immunostaining method using swine anti-rabbit alkaline phosphatase conjugate at a dilution of 1 : 50 to demonstrate factor VIII related antigens.

The substrate reaction was developed using 5 mg Naphthol AS-BI phosphate (Sigma) dissolved in 200 μl dimethylformamide. This was then mixed with 10 ml of 0.1 M tris-HCl buffer (pH 8.2) and 10 mg fast Red TR salt (Sigma). Endogenous alkaline phosphatase was inhibited by levamisole (10^{-3} M). Incubation with the substrate lasted 20 min. Sections were then counterstained with Mayer's haematoxylin and mounted with glycerine jelly (Dako) or aquamount (BDH). Primary antibody was replaced by TBS or non-immune immunoglobulin of the same species (mouse or rabbit) to check for non-specific binding,

TABLE I Clinical characteristics of the 30 patients analysed in this study

Sex	M	13
	F	17
Age range (years)	34–85	
Original joint disease	RA	8
	OA	17
	Sec OA/CDH	2
	Sec OA/PT	2
	Sec OA/ON	1
Duration of the implant	16 months–22 years	
Joint involved	Hip	19
	Knee	11
Type of wear debris in sections	Metal	1
	UHMWPE	10
	Metal + UHMWPE	15
	Metal + UHMWPE + PMMA	4

RA – rheumatoid arthritis; OA – osteoarthritis; Sec OA/CDH – secondary OA/congenital dysplasia/dislocation of the hip; PT – post traumatic; ON – osteonecrosis; UHMWPE – ultra high molecular weight polyethylene; PMMA – polymethylmethacrylate

TABLE II Antibodies used in the study

Antibody	Dilution	Specificity	Type/Class	Source/Ref.
1.2 B6	1/10	ELAM-1	Culture supernatant/mouse IgG1	Dr D. O. Haskard RPMS-London
1.4 C3	1/10	VCAM-1	Culture supernatant/mouse IgG1	Dr D. O. Haskard RPMS-London
TS1/22 (CD11a)	1/10	LFA-1	Culture supernatant/mouse IgG1	Dr D. O. Haskard RPMS-London
ICAM-1	1/50	ICAM-1, 90KD membrane glycoprotein	Purified mouse IgG1	Genzyme UK
CD11b	1/50	C3bi receptor	Culture supernatant/mouse IgG1	Dako
EBM11	1/50	CD68/ macrophages	Culture supernatant/mouse IgG1	Dako
CD4	1/20	Helper T-cells	Culture supernatant/mouse IgG1	Dako
CD8	1/20	Suppressor T-cells	Culture supernatant/mouse IgG1	Dako
CD45RA	1/20	Naive T-cells	Purified ascites/mouse/IgG1	Serotec
CD45RO	1/50	UCHL-1/memory T-cells	Culture supernatant/mouse IgG1	Dako
Von-Willebrand factor	1/200	Factor VIII-related antigen	Purified rabbit antiserum	Dako

while the possibility of there being detection of endogenous alkaline phosphatase in endothelial cells was excluded by incubating one section from every case in the presence of substrate alone.

3. Results

Sections of the bone–implant interface from the 30 cases in this study showed a prominent macrophage and lymphocytic infiltrate with a variable number of FBGCs. All cases showed at least one type of particulate implant material within the cytoplasm of the phagocytic macrophages and FBGCs as well as in the extracellular matrix. The most frequently detected wear debris was UHMWPE with or without metal, which was observed at various amounts throughout the sections. Using polarization light, UHMWPE appeared as birefringent particles of different shape and size. Metal debris appeared as black particles and had more localized distribution within the sections compared to polyethylene.

The histological pattern of the cellular infiltrate correlated strongly with the amount and the level of migration of particulate debris from the surface adjacent to the implant/bone cement to the deeper layers at the bone side. All macrophages and FBGCs were identified with anti CD68 antibody (Fig. 1), whereas T cells were detected with anti CD3. The macrophages colocalized with the released wear particles. This was clearly represented in the interface membranes that consisted of fibrous stroma with few layers of macrophages on the implant side only, where particulate material is first released. The migration of particles to the deeper layers was accompanied by the development of new vessels and marked increase in the number of migrating perivascular macrophages and T-cells. Vascular growth was seen at higher rate in cases with complete granulomatous transformation and the presence of a significant number of FBGCs. T-cells formed a small proportion of the inflammatory infiltrate compared to the macrophages. Immunophenotypic analysis revealed that 60–70% of these T-cells are CD4 positive helper type and they belonged predominantly to the CD45 RO positive memory T-cell subset.

Staining of vascular endothelium with antibody to factor VIII related antigens indicated the presence of different types of vessels in a highly organized pattern. A consistent feature of this pattern was the presence of small proliferating vessels with a vascular lumen alongside the surface lining the implant, whereas larger vessels are more abundant in the deeper layers.

3.1. Expression of the adhesion molecules

Expression of ELAM-1 was restricted to vascular endothelial cells, whereas VCAM-1 and ICAM-1 were present on vessels as well as the lining cells and subsets of macrophages and FBGCs. The results are shown in Tables III and IV. The number of positive vessels was scored on serial cryostat sections stained for factor VIII as a general marker expressed by all types of vessels and those stained for the adhesion molecule. The total number of vessels positive for factor VIII was quantified in one section and the number of vessels expressing each of the adhesion molecules was counted in the adjacent section. The percentage of positive vessels was then determined.

ELAM-1 was observed in 21 cases on a variable number of vessels ranged from 5–90% of the total vessels. These vessels showed no distinct pattern of distribution. ELAM-1 was upregulated on vessels in cases with increased cellular infiltrate. A prominent lymphocytic and macrophage infiltrate was evident around ELAM-1 positive vessels. A strong positive correlation was found between the frequency of ELAM-1 expression and the increased metal particulate debris. In 11 out of 21 positive cases, 40–90% of total vessels showed ELAM-1 upregulation and that was in association with metallosis (Fig. 2). Such cases often showed granulomatous transformation with an increased number of FBGCs. Metal-debris-containing macrophages and FBGCs were particularly seen around ELAM-1 expressing vessels.

Induction of VCAM-1 on vascular endothelium was at a lower level than ELAM-1 in these cases. VCAM-1 expressing vessels were seen in six cases only and the number did not exceed 40% of the total

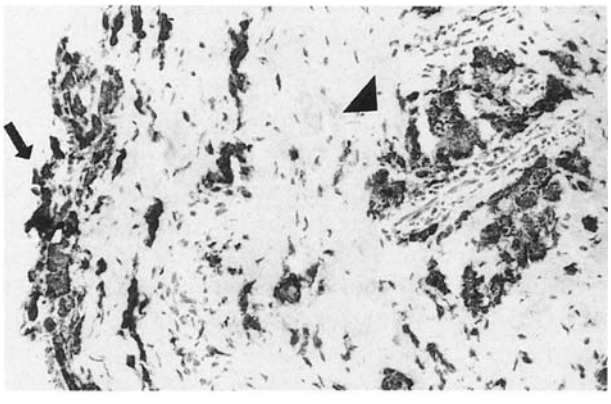

Figure 1 Section of the bone–implant interface stained for CD68 macrophage antigen. Macrophage infiltrate is seen at the lining layer on the implant side (arrow) and in the deeper layer around blood vessels (arrow head).

TABLE III Distribution of the adhesion molecules and the receptors on cells in the bone–implant interface

Adhesion molecule	Cell type	Distribution
ELAM-1	Vascular endothelium	Throughout the interface
VCAM-1	Vascular endothelium	Implant side and deeper layers
	Macrophages	Lining cells only
ICAM-1	Vascular endothelium	Deeper layers and the bone side
	Macrophage aggregates	Implant side only
	FBGCs	Implant side only
LFA-1	T-cells	Throughout the interface
CR3	Macrophages and FBGCs	Throughout the interface

TABLE IV The relative expression (%) of the adhesion molecules on vascular endothelium (number of cases in parentheses)

ELAM-1	VCAM-1	ICAM-1
5–10 (3)	5–40 (6)	5–30 (9)
20–30 (5)		40–75 (4)
30–60 (3)		
60–80 (4)		
80–90 (6)		

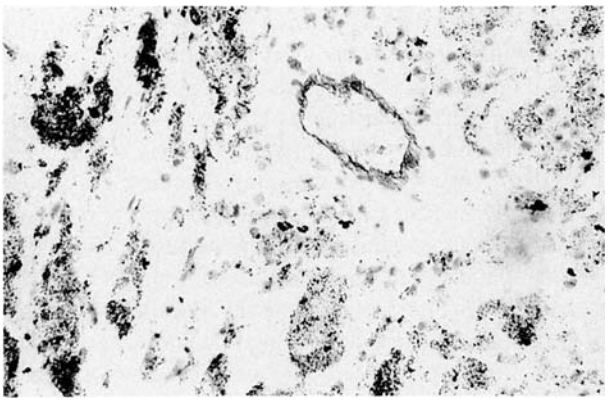

Figure 2 ELAM-1 expression on vascular endothelium in association with increased number of metal-debris-containing macrophages.

vessels. Interestingly three of these cases showed upregulation of both VCAM-1 and ELAM-1 that was correlated with the presence of metal particulate debris. Strong reactivity for VCAM-1 was frequently seen on the superficial cells of the lining layer at the implant side (Fig. 3). The majority of these cells expressed the CD68 macrophage antigen.

ICAM-1 expression was significantly higher than VCAM-1, and showed a distinct pattern of distribution. Upregulation of ICAM-1 on vascular endothelium was restricted to vessels on the bone side only and was observed in 13 cases. Although the number of positive vessels ranged from 5 to 75%, the majority of positive cases had a relatively low level of expression at about 30%. Vessels on the implant side were consistently negative for ICAM-1. In addition to endothelial cells ICAM-1 was strongly expressed on macrophage subsets that formed small aggregates, and a large number of multinucleated giant cells. These positive cells were predominantly found on the implant side only. ICAM-1 was also observed at high levels on all the chondrocytes in two samples of the interface undergoing chondroid metaplasia. Anti LFA-1 antibody reacted strongly with the T-cell infiltrate in all cases. CR3 was constitutively expressed at high levels on different macrophage subsets present in the interface (Fig. 4). Macrophage aggregates and a small number of FBGCs in the lining and sublining layers on the implant side exhibited simultaneous expression of both ICAM-1 and CR3. In comparison FBGCs throughout the interface showed a relatively lower level of expression of CR3 and lacked ICAM-1 expression.

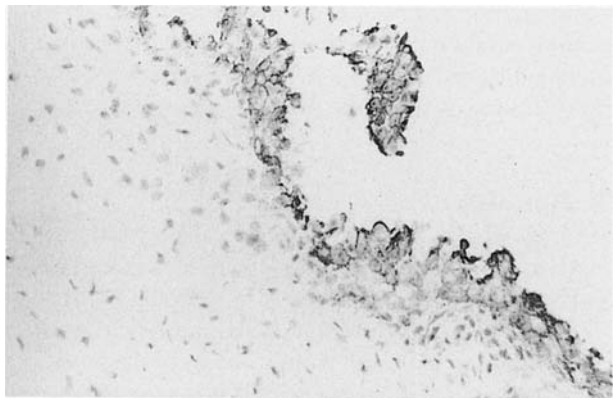

Figure 3 Lining cells on the implant side are frequently positive for VCAM-1.

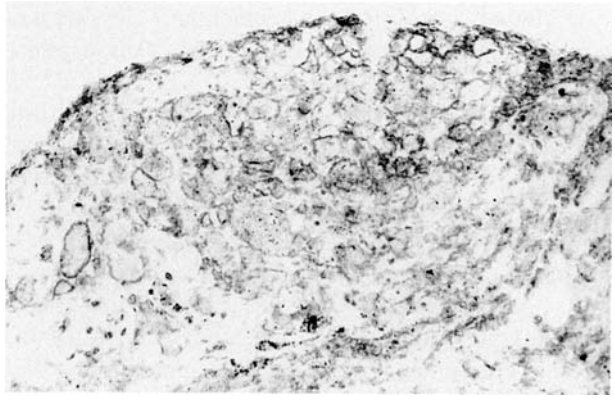

Figure 4 High level of expression of CR3, a receptor for ICAM-1 on all macrophages and some FBGCs throughout the interface.

4. Discussion

This study describes the histological pattern of vascular growth and the activation of endothelial cells in the bone–implant interface during aseptic loosening. The results indicate marked increase both in the number of newly formed vessels and the inflammatory infiltrate. These histological changes correlated with the release and presence of implant particulate material. Phagocytosis of such debris by locally present and migrating macrophages promotes activation as well as cell–endothelium and cell–matrix interactions. These cellular interactions are mediated by increased expression of cell adhesion molecules and their receptors.

The upregulated expression of ELAM-1, VCAM-1 and ICAM-1 on vessels in the interface suggests the potential involvement of at least three different pathways in the recruitment of macrophages and T-cells to these tissues. Interestingly the increased level of expression correlates strongly with that demonstrated in inflammatory synovitis and the rheumatoid synovium [28, 29]. In contrast normal synovium is known to lack or show weak expression of these adhesion molecules [30].

ELAM-1 expression on vascular endothelium (n = 21 cases) was more persistent than ICAM-1 and VCAM-1 (n = 13 and 6 cases, respectively). This continuous induction is usually related to sustained ac-

tivation of endothelial cells as previously described in other chronic inflammatory conditions such as psoriasis [31]. In the bone–implant interface we found significant increase in ELAM-1 expression in cases with metallosis ($n = 11$) of 21 positive cases. There was also co-expression of ELAM-1 and VCAM-1 in three of these cases. Metal particulate debris appear to be highly immunogenic in terms of their ability to induce the expression of adhesion molecules as well as activation of macrophages. We have recently reported the induction of IL-1 production by metal-debris-containing macrophages [17]. This cytokine is known to upregulate the expression of adhesion molecules on vascular endothelium [14, 15].

In this study ELAM-1 was immunolocalized in most of the cases that showed IL-1 production in our previous work. Metal release within the interface and the prolonged expression of ELAM-1 may contribute to recruitment of memory T-cells sensitized to metal. Upregulation of ELAM-1 and VCAM-1 on vascular endothelium has been reported in relation to hypersensitivity mediated immune reactions in allergic and toxic dermatitis [32, 33]. Our findings confirm and extend previous studies on contact hypersensitivity reactions to titanium or cobalt chrome in patients with orthopaedic implants [34, 35]. Furthermore, other workers have demonstrated the presence of metal wear debris in the serum of patients with failed prostheses [36]. The release of debris-containing phagocytes into the circulation and their contact with T lymphocytes in the blood may be critical in the recruitment of primed memory T-cells into the interface. This is supported by our observation that T-cells in the interface are frequently of the helper, memory type CD4, CD45RO positive.

The lack of VCAM-1 on vascular endothelium in the majority of the cases suggests a limited contribution in the recruitment of inflammatory cells. In contrast ICAM-1 mediated pathway of lymphocyte/macrophage adhesion is evident in the interface and is consistent with our finding of the increased expression of LFA-1 on T-cells and CR3 on macrophages, particularly in the perivascular areas. These two receptors are ligands of ICAM-1 [25, 26]. Expression of ICAM-1 and VCAM-1 in the bone–implant interface was not restricted to vascular endothelium and was more prominent in the lining cells on the implant side. Various other studies have also demonstrated ICAM-1 and VCAM-1 upregulation on different cells including fibroblasts, macrophages, dendritic reticulum cells of lymphoid follicles, and type A and B synoviocytes in inflammatory synovitis and rheumatoid arthritis [28, 29, 37, 38]. The expression of these adhesion molecules or their receptors on subsets of macrophages and FBGCs on the implant side of the interface may have an important role in maintaining a prolonged immune response at sites of particulate debris release. ICAM-1 has been shown to regulate immune functions requiring cell–cell contact [39]. Intercellular adhesion may also regulate cell differentiation which is mediated by cytokines and growth factors released by phagocytic macrophages. In addition recent work provide evidence that some adhesion molecules or their ligands may act as receptors for a variety of extracellular matrix proteins including fibronectin, collagen and laminin [40, 41].

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