JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 9 (1998) 849–853

Direct activation of mast cells by prosthetic biomaterial particles

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IL-4 is a mast cell and T cell produced immune cytokine that is important in the regulation of macrophage function. IL-4 has also been implicated in the induction of foreign body giant cell formation. In patients with long-term joint prostheses, a localized granulomatous inflammation develops in periarticular tissues and other organs where phagocytosis of particulate material from various prosthetic components takes place. In this study we used the inflammatory lesions of the bone–implant interface as a model to investigate the possible production, the frequency and the cellular source of IL-4. 40 samples of the interface membrane obtained from 25 patients undergoing revision of clinically failed implants were analyzed by immunohistochemistry. Cryostat sections were labeled with specific monoclonal antibodies to mast cell products: IL-4, tryptase and the receptor c-kit (CD117). The study has identified a significant level of production of IL-4 by mast cells in all the cases analyzed. There was an apparent difference in the number of mast cells in relation to the histological variants of the interface. The increase in the number of mast cells and IL-4 production was more pronounced in cases with heavy macrophage infiltrate than those exhibiting a predominance of giant cells. The findings imply that the recruitment of mast cell and IL-4 expression precede the granulomatous reaction and may have a role in the induction of a number of immunopathological changes related to mast cell activation by biomaterial particles.

1. Introduction

Local and systemic immunopathological changes have been documented in patients with aseptic loosening of total joint replacements (TJR). These patients show the development of localized granulomatous inflammatory lesions in periarticular tissues at the site of the implant [1] as well as in other organs where seeding of particulate material released from various implant components takes place [2]. Furthermore, the major contribution of macrophages (Mϕ) and T cells as the effector cells in these processes, are well established [3]. However, clinical evidence points to diverse situations in which osteolytic lesions and damage to bone have been observed around well-fixed implants [4]. In addition, early aseptic loosening due to allergic reactions to metal or a constituent of bone cement have been reported [5]. Our histopathological analysis of the bone–implant interface from revision operations indicates that bone erosion and aseptic loosening may occur much sooner than the development of Mϕ and foreign body granulomatous reaction [6]. As we have previously demonstrated, the interface membrane in such cases exhibits features of a fibrous stroma with low level of the Mϕ and T cell inflammatory infiltrate. All these conditions suggest the contribution of other cellular components in the mediation/augmentation of inflammatory responses of different nature to those initiated by Mϕ and T cells. The mast cell is a potential candidate in this context because of its presence in abundance within bone/bone marrow. Mast cells have also been observed in tissues associated with fibrotic, chronic inflammatory and proliferative diseases [7, 8], therefore the precise identification of mast cells and their products within inflammatory lesions is important for assessing mast cell dependent biological responses. Among the important cytokines produced by mast cells as well as T cells is IL-4. This 20 KD glycoprotein/immune cytokine functions as a regulatory factor for the growth and/or differentiation of a number of haemopoietic cells including B cells, mast cells and antigen specific helper T cells [9–11]. IL-4 is also important for the regulation of monocyte/macrophage function in localized inflammatory sites [12]. Recently, two independent studies have implicated IL-4 as a potential inducer of fusion and foreign body giant cell (FBGC) formation [13, 14]. In both studies the assessment of the effect of IL-4 was based on an *in vitro* culture system utilizing

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bone marrow mononuclear cells. However, none of these reports has considered the possible contribution of mast cells as a primary source of IL-4. The aim of this study was to investigate whether direct contact of mast cells with biomaterial particles can induce activation of MC and mediator release. We used the bone–implant interface membranes retrieved during revision operations in patients with clinically failed total joint replacement as a model to assess the possible production of IL-4 in these tissues, the cellular source, and distribution pattern and frequency of IL-4 production.

2. Materials and methods

Forty specimens of the bone–implant interface membrane were obtained from 25 patients undergoing first or second revision of clinically failed TJR (Table I). The revision was performed for aseptic loosening that showed associated bone resorption at variable locations and severity. The exact location of the tissue removed, the condition of adjacent bone, the incidence of lytic lesions as well as details of the patients' primary joint disease were all recorded. The analysis was carried out on cryostat tissue sections of the interface membranes. Mast cells were characterized with monoclonal antibodies to IL-4, c-kit (CD117) and tryptase (AA1). Mϕ and FBGC were identified with CD68, CD13 and CD11b (C_3biR/CR3), and T cells with CD3. The source of the antibodies and their working dilutions are listed in Table II.

Immuno-localization of mast cell products and other cellular markers was performed using a three-step immunoalkaline phosphatase with biotin and streptavidin conjugates as described previously [15].

Cryostat sections, 5 μm thick, were fixed in a mixture of acetone–methanol (50/50) at −20 °C for 10 min and washed with 0.05 M Tris-HCl buffered saline pH 7.6 (TBS). The tissue sections were then incubated with the optimum dilution of each antibody for 1 h at room temperature. This was followed by 1 h incubation with biotinylated horse anti-mouse IgG. Binding was achieved by a 1 h third incubation with streptavidin alkaline phosphatase complex. All the conjugated antibodies were purchased from Vector Laboratories, Peterborough, UK, and used at 1/100 dilution. Each incubation was followed by three washes with TBS of 5 min each. The enzyme color reaction was developed in 0.1 M Tris-HCl buffer (pH 8.2) containing Fast Red TR salt and the substrate Naphthol AS-BI phosphate (both from Sigma) dissolved in dimethylformamide. Levamisole (10^{-3} M) was added to the substrate solution as an inhibitor of endogenous alkaline phosphatase. The sections were washed with TBS after 20 min incubation. This was followed by counterstaining with Mayer's haematoxylin, blueing in water and mounting in glycerine jelly (Dako) or aquamount (BDH). Negative controls to check for non-specific binding included replacing the primary antibody with (1) TBS only, or (2) non-immune immunoglobulin from the same species as the first antibody used at the same concentration.

TABLE I Type of specimen and patient details

Bone–implant interface membranes (from revision operations of clinically failed total joint replacements)	40
No. of patients studied	25
Males	7
Females	18
Underlying joint disease	
RA	10
Primary or secondary OA	15
Joint revision	
THR	18
TKR	7
RA synovial membranes	10
OA synovial membranes (from hip or knee replacement operations)	15

TABLE II Primary monoclonal antibodies used in this study

Antibody	Dilution	Source
IL-4	1/50	Genzyme, UK
c-kit (CD117/SCFR)	1/200	Novocastra Laboraties
AA1 (MC Tryptase)	1/200	Dako
CD68	1/100	Dako
CD11b (C_3biR/CR_3)	1/100	Dako
CD13	1/100	Dako
CD3	1/20	Dako

3. Results

Serial tissue sections were examined for the presence of immunoreactive cells with the antibodies used. The number of positive cells was counted in ten high-power fields (HPF) using X20 objective, and the mean value was then estimated. Mast cells were identified by their positive membrane labeling for the receptor c-kit, a specific marker of mast cells and intense cytoplasmic staining for the serine proteinase tryptase, an exclusive product of these cells. The presence of diffuse extracellular staining for this enzyme is indicative of mast cell degranulation. Mϕ and FBGC were characterized by membrane staining for CD68 and CD13, whereas T cells were immunolocalized with CD3 Mab.

The extent of the inflammatory infiltrate and the number of FBGC in the interface membranes varied significantly between the cases but correlated with the amount of implant particulate wear debris detected within the sections (Fig. 1). The average number of IL-4 producing mast cells in the bone–implant interface and the RA and OA synovium was comparable to the number of tryptase (AA1) and c-kit positive mast cells. Immunostaining also revealed that these tissues exhibit a relatively identical distribution pattern of IL-4 expressing mast cells in the context of the presence of these cells at the perivascular areas, and in association with the increased level of the inflammatory infiltrate (Table III). Mast cells were less abundant in the lining layer of the interface membranes (at the implant side) compared to the superficial synovial lining in the RA or OA synovial membranes analyzed. Correlation of the number of IL-4 positive mast cells

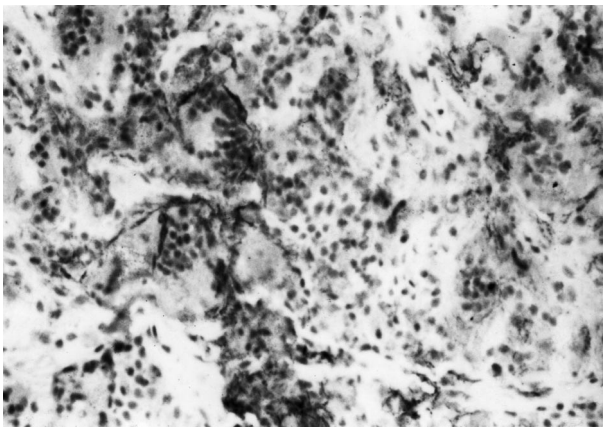

Figure 1 Giant cells within the granulomatous reaction are characterized by strong membrane staining for CD13. Femoral interface from revision of TKR in a patient with RA ×300.

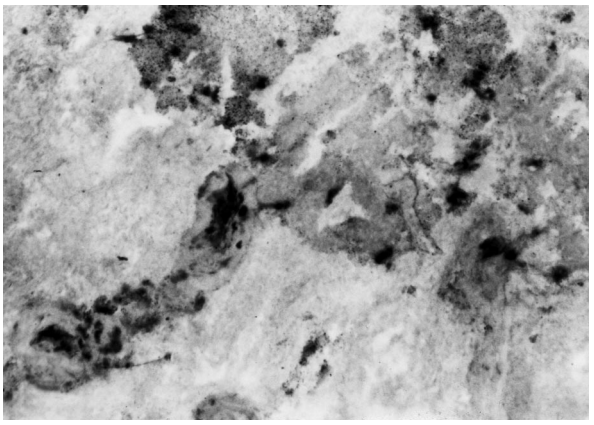

Figure 2 Immunolocalization of IL-4 in the mast cells within the fibrous stroma of the bone–implant interface distant from the Mϕ and T cell inflammatory infiltrate. Third revision of THR in a patient with RA ×300.

TABLE III Quantification of IL-4 positive MC in tissue sections of the bone–implant interface and the RA synovial membranes

Type of specimen	Range and (mean) no. of IL-4 $^{+}$MC/20 HPF		
	Lining layer	Areas of inflammatory cell infiltration	Fibrous stroma
Bone–implant interface	2–4(3)	10–35(23)	10–29(18)
RA synovium	2–5(4)	5–20(14)	7–25(17)
RA cartilage–pannus junction	2–4(3)	4–20(11)	–

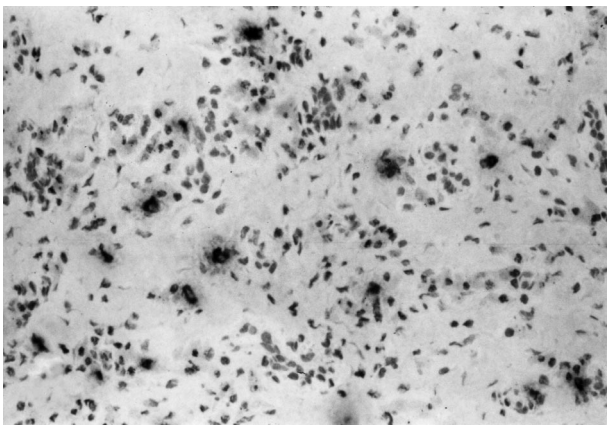

Figure 3 Staining of IL-4 within mast cells in a section of acetabular interface from revision of THR. Note the increase in the number of perivascular mast cells within the inflammatory infiltrate ×260.

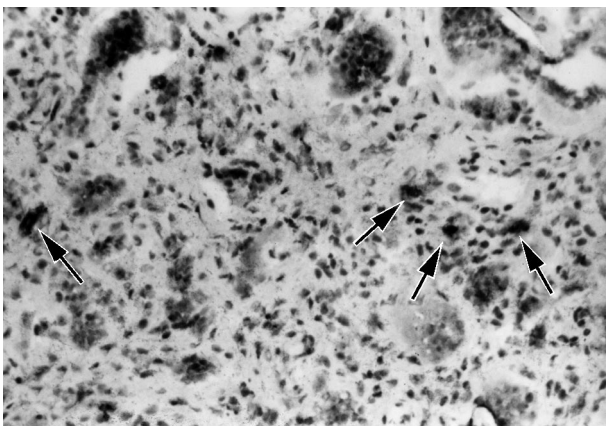

Figure 4 A small number of IL-4 positive mast cells was observed among the terminally differentiated foreign body giant cells, tibial interface ×200.

with the histological variants of the interface membrane demonstrated that the presence of mast cells and the induction of IL-4 production precede the formation of the granulomatous inflammatory lesions. This was consistent with the observation of a noticeably increased number of these cells within the fibrous stroma of the interface membrane adjacent to bone surfaces and in association with the Mϕ inflammatory infiltrate (Figs 2 and 3). Finally, immunostaining showed a reduction in the number of mast cells in cases in which FBGC predominated (Fig. 4). Interestingly five specimens of the interface membranes that showed significant Mϕ fusion and FBGC formation, but low mast cell counts were retrieved from patients whose primary joint disease was rheumatoid arthritis. These particular specimens also showed membrane labeling for IL-4 on a proportion of the T lymphocytes identified adjacent to metal/polyethylene containing Mϕ subsets. Similarly, a proportion of the T cells found within the RA synovial membranes obtained from replacement operations also showed membrane staining for IL-4.

4. Discussion

This study demonstrates the induction of mast cell IL-4 expression following the exposure/contact with biomaterial particles. The findings also imply that mast cells are a significant source of IL-4 production in the two models of chronic inflammatory conditions analyzed. The precise role of IL-4 in inflammatory reactions is still a subject of controversy. Hart *et al.* [16] reported anti-inflammatory effect of IL-4 and its suppressive activity on the production of inflammatory cytokines. From their data it has been suggested

that IL-4 may have a possible therapeutic role in the management of chronic inflammatory diseases. In contrast, other investigators have implicated IL-4 in pro-inflammatory immune responses [17] particularly with regard to its ability to stimulate Mϕ fusion and FBGC formation, the induction of adhesion molecules such as VCAM-1 and the differentiation of T helper 2 (Th2) cells [18, 19]. This T cell subset is known to have a role in hypersensitivity reactions and chemically induced autoimmunity in experimental animals [20]. We suggest that IL-4 expression in the mast cell at the bone–implant interface membrane and the RA synovia may be responsible for a wide spectrum of mast cell-associated pathological changes that could be either augmented or inhibited by other mediators known to be produced at the site of these two inflammatory lesions. The results in this study favor the role of mast cells in the early immuno-pathological processes related to bone erosion. This interpretation is supported by a number of previous and more recent observations of the presence of large numbers of mast cells in conditions associated with excess bone resorption. Examples include pathological osteopenia in cases with mastocytosis [21], the bone marrow of post-menopausal women [22] and rheumatoid arthritis [23]. The *in situ* immunolocalization of mast cells in tissue sections in this study has allowed a precise quantification of the mast cells present within the inflammatory erosive lesions of the bone–implant interface. In addition, it has facilitated the assessment of the number of these cells in the context of the histological changes and cellular transformation of wear-debris containing phagocytes into bone-resorbing giant cells. Moreover, it was also possible to characterize these changes in relation to IL-4, protease (tryptase) and cell membrane receptor (c-kit) profile of mast cells in these tissues. As demonstrated in this analysis, a marked increase in the number of these cells accompanied the presence of heavy inflammatory infiltrate that shows features of cellular fusion and giant cell formation. In these cases, mast cells showed a remarkable increase in the fequency of IL-4 expression as well as exocytosis (degranulation) which was evident in the demonstration of extracellular immunoreactivity for tryptase in the sections. In contrast, the cases that showed obvious histological transformation and predominance of FBGC exhibited a small number of mast cells and a lower level of IL-4 and tryptase, suggesting dissolution of the cytoplasmic granules following exocytosis. The inactivated status of mast cells in these cases was also apparent from our observation of weak expression of the c-kit membrane receptor on these cells which is possibly related to internalization of the receptor following degranulation.

The colocalization of the Mϕ inflammatory infiltrate together with the increase in mast cell number imply close interaction between these two potential cell types. Such interaction could have various implications. Based on a recent report that mast cells are a major source of leukocyte-chemotactic peptides (MCP-1) [24] the role of these cells in the early chemotaxis and accumulation of inflammatory cells at sites of wear-debris release, cannot be ruled out. In addition to the role of IL-4 in promoting Mϕ fusion and FBGC formation, the substantial increase in the level of production of this cytokine by mast cells in the interface could contribute to the formation of the fibrous stroma of these membranes. This conclusion is consistent with previous reports of the expression of IL-4 receptors by fibroblasts [25], and the stimulatory effect of IL-4 in the regulation of extracellular matrix biogenesis during normal wound healing and in pathological fibrosis [26]. Further studies employing direct exposure of mast cells to metal or polyethylene particles are underway within our group, which will provide more information regarding activation, degranulation and interaction of mast cells with different T cells subsets.

In conclusion, our data demonstrate that the accumulation of implant wear particles at sites of prosthetic joints could enhance the recruitment of mast cells. In addition, mast cells appear to be a primary source of IL-4 in these tissues. The possible mechanisms by which IL-4 could augment local and systemic immunopathological changes were highlighted.

Acknowledgment

This work was supported by the EPSRC, UK, through the Interdisciplinary Research Centre (IRC) in Biomedical Materials. We wish to thank the orthopaedic surgeons at the Royal Free Hospital and the Royal London Hospital for providing the tissue specimens used in the study. We are also grateful to Miss Lynne Walters for excellent secretarial help.

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Received 7 May
and accepted 1 June 1998