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Interleukin 15 production by macrophages in the implant interface membrane of aseptically loosened joint replacements

P. A. REVELL*, S. E. JELLIE
Osteoarticular Research Group and IRC in Biomedical Materials, Department of Histopathology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, UK
E-mail: parevell@rfhsm.ac.uk

The T lymphocytes are cells involved in immunologically mediated hypersensitivity reactions. They are found in the cellular infiltrate present in the interface membrane of aseptically loosened joint prostheses. Activation of these cells has been demonstrated but not the important cytokine, interleukin 2 (IL2) required for such activation. This study describes the localization of IL15 and its mRNA in macrophages in interface membranes associated with activated T lymphocytes which are proliferating (as is evident from HLA-DR and Ki67 expression, respectively). The findings provide further evidence for immune mediated processes in aseptic loosening of orthopaedic implants because IL15 has similar activities to IL2 in T lymphocyte activation.

1. Introduction

Examination of tissue forming the interface membrane of aseptically loosened prosthetic joints has shown the presence of an inflammatory cellular reaction in response to particulate wear debris from the bearing surfaces of the joint [1]. The literature relating to the particles detectable by light microscopy has been reviewed recently [2]. It is now recognized that most particles are beyond the resolution of the light microscope being less than 1 μm in size [1, 3–5]. Increasing evidence suggests that these sub-micrometer sized particles play an important part in the bone loss (osteolysis) occurring adjacent to implants [6]. The normal defence of the body to particulate foreign materials involves cells of the macrophage system which engulf them. Macrophages and multinucleate giant cells (MNGCs), which are closely related, are found in the interface tissues where there is aseptic loosening and osteolysis. The inflammatory mediators produced by these macrophages include cytokines and nitric oxide which, in turn, play a role in stimulating resorption of bone by macrophages and osteoclasts [1, 7–10].

There are usually lymphocytes of the T cell subset intermingled with the debris-containing macrophages [11, 12] and these cells are more numerous where there is metal debris, prompting the suggestion that there is a metal sensitivity reaction occurring in some cases of aseptic loosening [11]. Although these T lymphocytes are activated [13, 14], the cytokine normally responsible for such activation in immune processes (interleukin 2, IL2) has not been demonstrated in interface membranes. The recently described cytokine, interleukin 15 (IL15) has similar activities to IL2 and shares the IL2 receptor (IL2-R) on lymphocytes as well as having a receptor of its own [15, 16]. The presence of IL15 and its messenger RNA (mRNA) was sought therefore in interface membranes by immunohistochemical and *in situ* hybridization techniques.

2. Materials and methods

Samples (30 in all) of interface membrane from total hip replacements were obtained at the time of revision surgery for aseptic loosening. Microbiological culture in all cases showed the absence of infection. The samples were snap frozen in isopentane-cooled liquid nitrogen and stored at $-70\,^{\circ}C$ before use. Frozen sections were cut at 5 μm thickness using a Reichert Jung cryostat, placed on poly-*L*-lysine coated slides and air dried for 2 h. The sections were stained using the alkaline phosphatase/streptavidin method. They were fixed in cold acetone for 15 min, washed three times for 5 min each in phosphate buffered saline (PBS) then incubated with 1 : 20 dilution of universal blocking serum (Vector) + 5% bovine serum albumin (BSA) in PBS for 30 min. The serum was blotted out, then a further blocking avidin/biotin (Vector kit) stage was performed, with an avidin blocking solution followed by a biotin blocking solution for 15 min each.

* Author to whom all correspondence should be addressed.

The sections were washed thoroughly between stages of avidin/biotin blocking. Antibodies to IL15 (1 : 50, mouse monoclonal antibody, Genzyme), IL2 (1 : 20, goat polyclonal antibody, Autogen Bioclear), IL2-R (1 : 20, CD25, Seralab), CD3 (1 : 20, rabbit polyclonal antibody, DAKO) and CD68 (1 : 100, EBM11, Dako) were applied for 1 h. After washing for three times for 5 min each in PBS, appropriate biotinylated secondary antibodies (1 : 50 dilution) were applied to the sections which were then incubated for 30 min. After three 5 min washes in PBS, sections were incubated with alkaline phosphatase/streptavidin (1 : 100) for 30 min and the reaction product developed with the alkaline phosphatase substrate kit (Vector).

Staining for HLA-DR and with the proliferation marker Ki67 was performed on ten samples using a fluorescent labeling method. Sections were prepared, acetone fixed and blocked as above, then incubated with anti-HLA-DR (1 : 50, rat polyclonal antibody, Dako) or Ki67 (1 : 30, sheep polyclonal antibody, Binding Sites Limited) for 1 h. The sections were washed, incubated with the appropriate (species specific) biotinylated secondary antibody and further washed before alkaline phosphatase/streptavidin (1 : 100) was applied for 30 min. The reaction product was developed with the alkaline phosphatase substrate kit (Vector).

In situ hybridization was performed using a cocktail of digoxigenin-labeled oligonucleotide probes to three separate sequences of IL15 mRNA (Boehringer) on formalin-fixed paraffin wax embedded tissue sections. After dewaxing and rehydration, sections were rinsed in 0.2 M HCl followed by 0.05 M Tris HCl (pH 7.4). Permeabilization with proteinase K (5 $\mu g\,ml^{-1}$) at 37 °C for 1 h was followed by washing in PBS with post-fixation on 0.4% paraformaldehyde/PBS for 20 min at 4 °C. Following further washing in DEPC distilled water, prehybridization was performed in a humidifying chamber at 37 °C for 1 h. The sections were hybridized at 37 °C overnight. Post-hybridization washes in Tris buffer (three times for 10 min washes) at 37 °C were followed by one wash at room temperature, and 0.1% Triton X-100 for 15 min at room temperature. Detection of digoxigenin-labelled probe was performed with anti-digoxigenin antibody labeled with alkaline phosphatase (1 : 250; Boehringer) and an alkaline phosphatase substrate kit (Vector Red).

Placenta, a tissue rich in IL15, was used as a positive control for both immunohistochemistry and *in s*[e]*itu* hybridization studies. Standard blocks of tonsil in which staining patterns are well known for all the other markers, were used as positive controls for these antibodies.

3. Results

Intracellular interleukin 15 was demonstrable in macrophages and multinucleate giant cells of 26 out of 30 samples. The IL15 containing cells were present in the synovium-like layer of macrophages adjacent to the implant (Fig. 1). There were also IL15 positive cells distributed deep in this layer through the fibrous

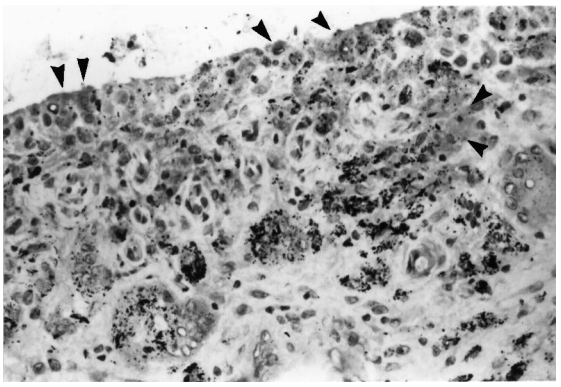

Figure 1 Synovium-like layer and deeper infiltrating cells of the interface tissue showing the presence of numerous IL15 containing macrophages immunostained with monoclonal antibody (arrow heads indicating some of these) and an IL15 positive giant cell (right). Immunohistochemistry; alkaline phosphatase method.

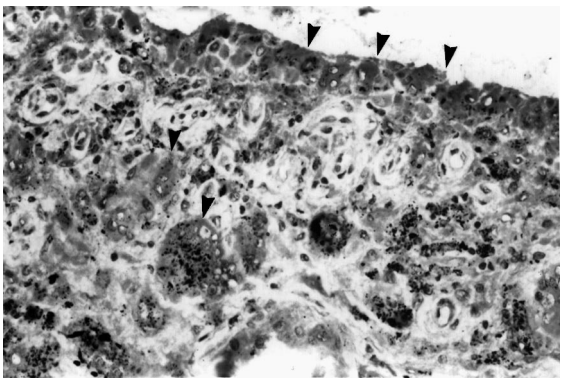

Figure 2 Adjacent section to that shown in Fig. 1, showing cells labeled with monoclonal antibody against CD68, a macrophage marker. Immunohistochemistry; alkaline phosphatase.

tissue of the interface membrane. Staining of serial sections with CD68 confirmed that the IL15-containing cells were macrophages (Fig. 2). The numbers of such cells varied but they were a prominent feature in eight of the cases.

Staining for IL2 showed the absence of this cytokine from all the samples from all the cases. Small numbers of lymphocytes showed surface staining for IL2-R.

There were plentiful T lymphocytes present in all but one case, as was evident from positive staining with CD3 monoclonal antibody (Fig. 3). In the sample of ten cases in which an activation marker was used, a proportion of macrophages and T lymphocytes were activated, as shown by the expression of HLA-DR on the cell surface membrane. A small percentage of T lymphocytes were proliferating, showing Ki67 staining of their nuclei (Fig. 4).

In situ hybridization studies showed the presence of IL15 mRNA in macrophages. These cells were present both near the implant (synovium-like) layer and on the bone side of the interface membrane. Their distribution was similar to that of cells immunostained with the antibody to IL15 indicating that both mRNA and the protein are detectable within macrophages in these

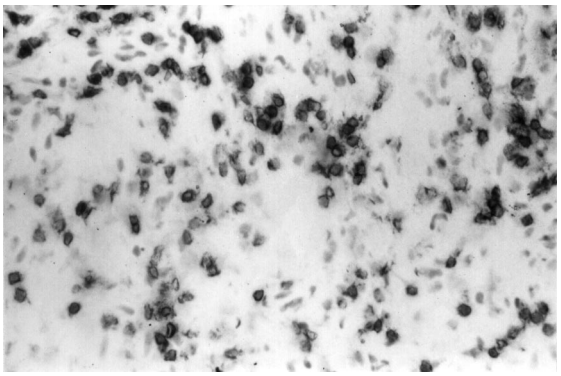

Figure 3 Interface membrane tissue showing the presence of numerous T lymphocytes labeled with monoclonal antibody against CD3. Immunohistochemistry; alkaline phosphatase.

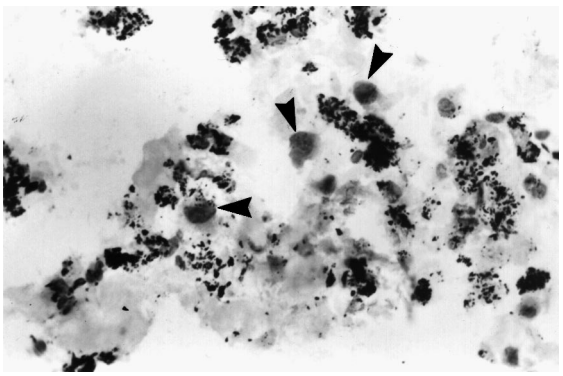

Figure 4 High power view of macrophages containing particulate metal debris (black granules). There are lymphocytes present which show nuclear staining with monoclonal antibody Ki67 (arrow heads). These are proliferating lymphocytes. Immunohistochemistry; alkaline phosphatase.

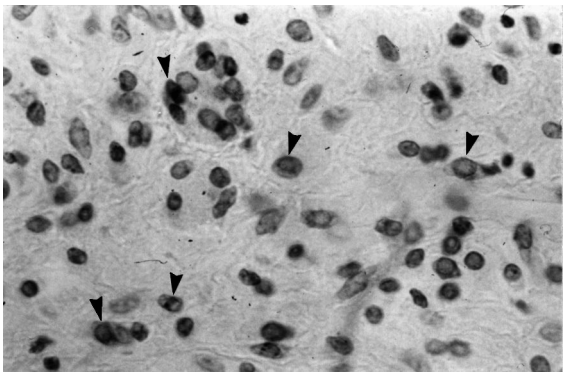

Figure 5 *In situ* hybridization to show macrophages expressing IL 15 mRNA in their nuclei. There are numerous positive cells present; five have been marked with arrow heads. Digoxigenin method; alkaline phosphatase.

cases. The mRNA could be localized to the nuclei of a proportion of macrophages (Fig. 5).

4. Discussion

Sensitivity reactions to metals used in orthopaedic implants have been recognized on clinical grounds in the past [17–19]. Fairly recently, the question of sensitization to titanium has been raised [11] and a further example of proven sensitivity to titanium has been reported [20]. It has been demonstrated that the T cells are arranged round blood vessels in the interface membrane [14], that they are primed cells with immune memory, because they show surface expression of CD45RO [21, 22], and that they are activated expressing HLA-DR [13, 14], a finding confirmed in the present study.

Further evidence comes from the expression of E-selectin, an adhesion molecule, by endothelial cells in newly formed blood vessels in the regions of the interface related to metal debris [22, 23]. This molecule is known to mediate T cell adhesion to endothelial cells and their migration through the vessel wall in contact/allergic hypersensitivity reactions [24]. It thus seems a possibility that a T cell mediated hypersensitivity reaction may be occurring in the interface tissues of some cases in association with the presence of metal debris. Others have described the reaction to titanium debris but considered the inflammatory mechanism to be no different from that occurring with any other particulate debris, considering the T lymphocytes to be of no functional significance [25–28]. Failure to demonstrate the presence of IL2 or its receptor (IL2-R) on such activated T lymphocytes has suggested that this opinion may be true. This finding is repeated here; however, the significant amount of IL15 present in macrophages and the fact that the mRNA for this cytokine can also be demonstrated, is evidence that a true immune response may be taking place. IL15 uses the IL2-R and has a receptor of its own, not sought in this study. It may be acting as an alternative to IL2 in the particular immune reaction occurring in interface membranes. Its presence as an activator of T lymphocytes and their proliferation is in keeping with the other findings of this study.

That there may be a different cellular mechanism in response to polyethylene compared with metal debris, is suggested by other recent findings. In histomorphometric studies of the type of cell found at the resorbing surfaces of bone adjacent to wear particle-containing cellular infiltrates, macrophages were found to be present to a significantly greater extent than osteoclasts [29]. Moreover, when the type of particle was examined in relation to areas of osteolysis and compared with areas where there was no osteolysis, macrophages were found to be significantly increased in the presence of polyethylene debris, whereas osteoclasts were the cells found at a significantly higher level in relation to metal debris [30]. The possible differences in mechanisms in relation to different types of particle are the subject of on-going studies.

Acknowledgments

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References

1. P. A. REVELL, N. AL-SAFFAR and A. KOBAYASHI, *Proc. Inst. Mech. Eng.* **211H** (1997) 187.
2. J. A. SAVIO, L. M. OVERCAMP and J. BLACK, *Clin. Mater.* **15** (1994) 101.

3. K. J. MARCEVICIUS, T. W. BAUER, J. T. McMAHON, S. A. BROWN and K. MERRITT, *J. Bone Joint Surg.* **76A** (1994) 1964.
4. A. S. SHANBHAG, J. J. JACOBS, T. T. GLANT, J. L. GILBERT, J. BLACK and J. O. GALANTE, *ibid.* **76B** (1994) 60.
5. P. CAMPBELL, S. MA, T. SCHMALZRIED and H. C. AMSTUTZ, *J. Biomed. Mater. Res.* **28** (1994) 523.
6. T. P. SCHMALZRIED, M. JASTY and W. H. HARRIS, *J. Bone Joint Surg.* **74A** (1992) 849.
7. S. R. GOLDRING, A. L. SCHILLER, M. ROELKE, C. M. ROURKE, D. A. O'NEILL and W. H. HARRIS, *ibid.* **65A** (1983) 575.
8. W. P. JIRANEK, M. MACHADO, M. JASTY, D. JEVSEVAR, H. J. WOLFE, S. R. GOLDRING, M. J. GOLDBERG and W. H. HARRIS, *ibid.* **75A** (1993) 863.
9. N. AL-SAFFAR, H. A. KHWAJA, Y. KADOYA and P. A. REVELL, *Am. J. Clin. Pathol.* **105** (1996) 628.
10. E. MOILANEN, T. MOILANEN, R. KNOWLES, I. CHARLES, Y. KADOYA, N. AL-SAFFAR, P. A. REVELL and S. MONCADA, *Am. J. Pathol.* **150** (1997) 881.
11. P. A. LALOR, P. A. REVELL, A. B. GRAY, S. WRIGHT, G. T. RAILTON and M. A. R. FREEMAN, *J. Bone Joint Surg.* **73B** (1991) 25.
12. N. AL-SAFFAR, P. A. REVELL and J. A. SACHS, *J. Pathol.* **169** (1993) Suppl. 105A.
13. P. A. REVELL, P. A. LALOR and N. AL-SAFFAR, in "Implant Retrieval Symposium of the Society of Biomaterials", St Charles, IL, USA, September 1992.
14. N. AL-SAFFAR and P. A. REVELL, *Br. J. Rheumatol.* **33** (1994) 309.
15. K. H. GRABSTEIN, J. EISENMAN, K. SHANEBECK, C. RAUCH, S. SRINIVASAN, V. FUNG, C. BEERS, J. RICHARDSON, M. A. SCHOENBORN, M. AHDIEH, I. JOHNSON, M. R. ALDERSON, J. D. WATSON, D. M. ANDERSON and J. G. GIRI, *Science* **264** (1994) 965.
16. J. D. GIRI, M. AHDIEH, J. EISENMAN, K. SHANEBECK, K. GRABSTEIN, S. KUMAKI, A. NAMEN, L. S. PARK, D. COSMAN and D. ANDERSON, *EMBO J.* **13** (1994) 2822.
17. E. M. EVANS, M. A. R. FREEMAN, A. J. MILLER and B. VERNON-ROBERTS, *J. Bone Joint Surg.* **56B** (1974) 626.
18. M. W. ELVES, J. N. WILSON, J. T. SCALES and H. B. S. KEMP, *Br. Med. J.* **4** (1975) 376.
19. M. K. D. BENSON, P. G. GOODWIN and J. BROSTOFF, *ibid.* **4** (1975) 374.
20. C. P. CASE, V. G. LANGKAMER, C. JAMES, M. R. PALMER, A. J. KEMP, P. F. HEAP and L. SOLOMON, *J. Bone Joint Surg.* **76B** (1994) 701.
21. P. A. REVELL and N. AL-SAFFAR, in "Failure of Joint Replacement. A biological, mechanical or surgical problem?" edited by S. Downes and M. Dabestani (Institute of Orthopaedics, London, 1994) p. 89.
22. N. AL-SAFFAR, P. A. REVELL and J. A. SACHS, *Trans. Soc. Biomater.* **19** (1993) 193.
23. N. AL-SAFFAR, J. T. L. MAH, Y. KADOYA and P. A. REVELL, *Ann. Rheum. Dis.* **54** (1995) 201.
24. P. NORRIS, R. N. POSTON, D. S. THOMAS, M. THORNHILL, J. HAWK and D. O. HASKARD, *J. Invest. Dermatol.* **96** (1992) 763.
25. H. J. AGINS, N. W. ALCOCK, M. BONSAL, E. A. SALVATI, P. D. WILSON, P. M. PELLUCI and P. G. BULLOUGH, *J. Bone Joint Surg.* **70A** (1988) 347.
26. A. V. LOMBARDI, T. H. MALLORY, B. K. VAUGHAN and P. DROUILLARD, *ibid.* **71A** (1989) 1337.
27. S. SANTAVIRTA, Y. T. KONTINNEN, V. BERGROTH, A. ESKOLA, K. TALLROTH and T. S. LINDHOLM, *ibid.* **72A** (1990) 252.
28. S. SANTAVIRTA, Y. T. KONTINNEN, V. HOIKKA and A. ESKOLA, *ibid.* **73B** (1991) 38.
29. Y. KADOYA, P. A. REVELL, N. AL-SAFFAR, A. KOBAYASHI, G. SCOTT and M. A. R. FREEMAN, *J. Orthop. Res.* **14** (1996) 473.
30. *Idem.*, *Clin. Orthop. Rel. Res.* **340** (1997) 118.

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