

CYTOKINE AND MATRIX METALLOPROTEINASE EXPRESSION BY SUBCHONDRAL MULTINUCLEATED CELLS OF THE RHEUMATOID LESION.

DE. Woolley and LC. Tetlow

University Department of Medicine, Manchester Royal Infirmary, Oxford Road, M13 9WL. UK

Introduction.

Osteoclasts and chondroclasts have been implicated in bone and cartilage degradation of subchondral sites of the rheumatoid lesion [1]. Specific cytokines and proteinases are reportedly associated with osteoclast differentiation and function. This study has examined the expression of three proinflammatory cytokines and four matrix metalloproteinases (MMPs) by multinucleated cells in situ using immunohistochemistry.

Methods.

Cartilage/pannus junctions with underlying bone were sampled from rheumatoid knee specimens obtained from joint replacement surgery. Tissues were fixed in Carnoy's, processed to paraffin wax and tissue sections cut at 5 μ m. Twenty three specimens containing multinucleated cells were used for an immunohistochemical (IHC) study of cytokine (IL-1 β , TNF α and IL-15) and MMP (MMP-1, -8, -9, -13) distributions. Sheep polyclonal antibodies to MMP-1, -9 -13, a monoclonal antibody to MMP-8 and goat polyclonal antibodies to the cytokines were used with alkaline phosphatase-conjugated avidin-biotin complex for IHC [2]. Osteoclastic cells were also identified by acid phosphatase (AP) staining as described [1]. Photomicrographs were taken using a Zeiss Photomicroscope III and Ektachrome 160T film.

Results.

A proportion of multinucleated cells were positively stained for interleukin-1 β (IL-1 β), interleukin-15 (IL-15) and tumour necrosis factor- α (TNF α), this being more prominent than that for neighbouring cells. AP-stained osteoclasts were also shown to contain MMP-1, MMP-8, MMP-13 and MMP-9, but this was variable with some multinucleated cells appearing negative for these MMPs. Thus evidence is presented to show that multinucleated osteoclasts have the ability to produce these seven proteins in situ, but unlike AP staining which appeared to be a consistent feature, the cytokine and MMP expression was variable within the multinucleated cells of the same specimen.

Discussion.

The resorption cycle of osteoclasts is considered a multistep process. The variable expression of cytokines and MMPs may relate to stages of osteoclast differentiation/maturation, or to locally induced phenotypic changes in function [3]. The intracellular staining of TNF α , a potent factor for the induction of differentiation and bone resorption by osteoclasts [4], suggests an autocrine function. However, as yet it remains uncertain whether any of the visualised proteins have an extracellular function.

References.

1. Bromley M & Woolley DE (1984) *Arthritis Rheum.* 27: 968-97
2. Tetlow LC, Adlam DJ & Woolley DE. (2001) *Arthritis Rheum.* 44:585-594
3. Vaananen HK, Zhao H, Mulari M & Halleen JM. (2000) *J cell Sci.* 113:377-381
4. Azuma Y, Kaji K et al (2000) *J Biol Chem* 275: 4858-4864

Acknowledgements

This work was supported by the **Arthritis Research Campaign, UK**