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Osteochondritis dissecans

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Sir: Osteochondritis dissecans (OCD) is a common disease of unknown aetiology which particularly affects young people.[1] It generally represents an osseous lesion and a deficiency in vascular supply due to traumatic events, with secondary involvement of the overlying cartilage.[2] A certain genetic predisposition has also been hypothesized, but the causes are still debated.[3] Treatment is usually non-surgical for stable and surgical for unstable lesions, with the clinical results depending on many factors, including localization and size of the lesion, treatment and patient age. Generally, the disorder is characterized by the separation of one or more cartilage fragments from the subchondral bone and their release into the joint cavity.[4] In recent years, many investigators have focused their research on the biochemical events involved in the pathogenesis of joint diseases, but few studies have addressed the aetiology of OCD. Some authors have reported an increase in the catabolic activity of chondrocytes with enzymatic degradation of the extracellular matrix due to the action of metalloproteinases,[5] but further studies aimed at elucidating the mechanisms involved are needed. Apoptosis has been identified as one of the mechanisms associated with different processes observed in osteoarthritis (OA) and rheumatoid arthritis.[6] The damaged joint is the ideal milieu for apoptosis to occur, due to the simultaneous presence of mediators of inflammation such as cytokines, chemokines and reactive oxygen species.[7,8] It has been demonstrated in the early phases of OA that nitric oxide (NO) production may lead to chondrocyte apoptosis and that this phenomenon is particularly evident in the superficial zone.[9]

The aim of our study was to highlight the mechanisms of OCD, looking for apoptotic activity as one of the probable causes of pathology.

Cartilage samples were obtained from seven patients (mean age 25 years, range 18–34) who had undergone ankle arthroscopy for autologous chondrocyte transplantation. As controls, two biopsy specimens of healthy cartilage were obtained from two different multiorgan donors aged 27 and 32 who had no known history of arthritis or other joint pathology. Informed consent from the patients and approval by the ethics committee of the hospital were obtained. Cartilage samples were analysed histologically, immunohistochemically and by *in situ* cell death detection methods.

The OCD samples showed the typical morphology of hyaline articular cartilage, in which the different zones of cartilage were easily identifiable; the superficial cells appeared tangentially orientated to the surface, homogeneously distributed in the middle and arranged in columns in the deep zone. The presence of proteoglycans was confined to the middle and deep zones and collagen fibres were particularly evident in the superficial layer (Figure 1). The immunohistochemistry of type II collagen showed the presence of positive cells throughout the entire cartilage thickness, whereas type I collagen was negative. Caspase-3 enzyme, which belongs to a family of proteins responsible for the degradation of targeted cells to undergo apoptosis, showed diffuse positivity, particularly in the intermediate zone of cartilage of OCD samples. In the superficial layer of the samples analysed, we detected the presence of a large number of cells positive for both apoptosis (evaluated by terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling assay) and nitrotyrosine, a marker of NO production. The same cells were also immunopositive for nitrotyrosine, which is localized in the cytoplasm (Figure 2). This is not an unusual finding, since chondrocyte apoptosis has already been documented both *in vivo* and *in vitro* after a traumatic event, giving further support to the hypothesis of a mechanical injury as one of the aetiopathogenic factors in OCD.[10] One could hypothesize that, as a consequence of initial mechanical damage, cartilage suffers some microfissures, which allow the tissue to be more exposed to the synovial fluid. This causes the diffusion of oxygen and nutrients through cartilage. Chondrocytes, which are mainly anaerobic cells, in particular those of the

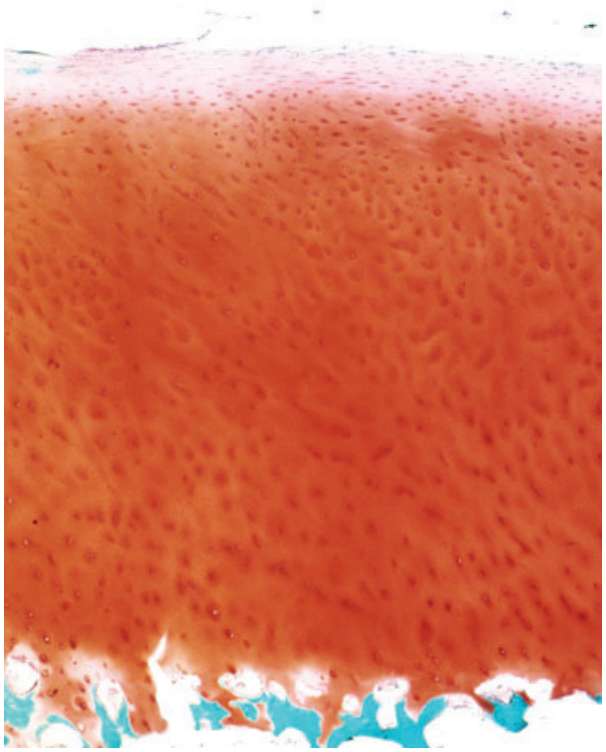

Figure 1. Safranin-O staining in an osteochondritis dissecans biopsy specimen shows the typical structure of hyaline cartilage.

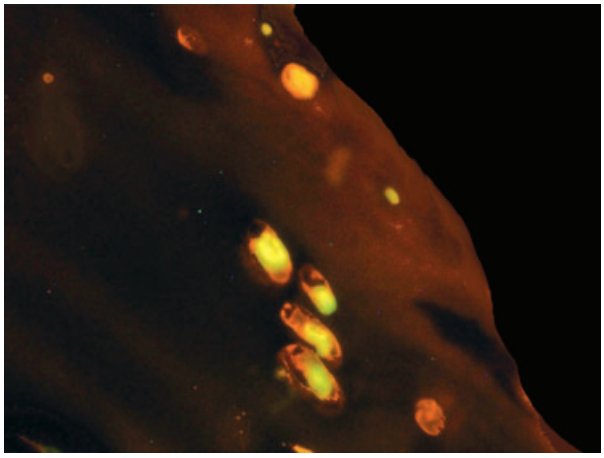

Figure 2. Cells positive for both apoptosis and nitrotyrosine are shown. The green stain indicates nuclei positive for terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling assay, while nitrotyrosine immunoreactivity in red is seen in the cytoplasm.

superficial layer, direct their metabolism to a catabolic pathway. This biochemical programme heralds a cartilage remodelling process which begins with the production of cytokines of the interleukin (IL)-1 family by resident cells and infiltrating inflammatory cells, and it is well known that IL-1 induces the biosynthesis of NO by articular chondrocytes.[11] NO may contribute to cartilage catabolism by causing free-radical damage of the matrix close to the chondrocytes and/or by acting intracellularly to trigger signalling events that result in further changes in gene expression.[12] At the end of this process many chondrocytes present in the upper layer of cartilage die by apoptosis.

Even if this study has demonstrated one of the mechanisms responsible for OCD, other mechanisms are certainly involved. However, the finding of an apoptotic process suggests that the use of pharmacological treatment may be appropriate for this pathology, which may be performed by local inhibition by different substances, such as cytokines and growth factors. Investigations are in progress to determine if other catabolic substances are involved in osteochondritis dissecans, such as synthesis and secretion of certain metalloproteinases.

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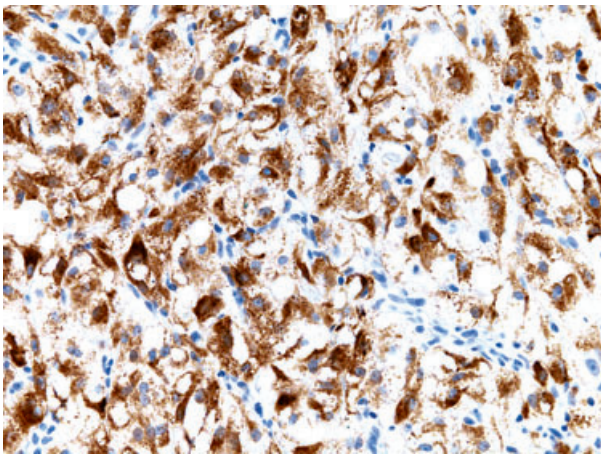

Figure 1. Tumour cells with central oval nuclei and abundant clear cytoplasm (H&E).

PEComas of the skin: more common in the lower limb? Two case reports

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Sir: Perivascular epithelioid cell neoplasms or PEComas are rare neoplasms which encompass angiomyolipomas, lymphangiomyomatosis, clear cell 'sugar' tumours (CCST) and clear cell myomelanocytic tumours (CCMMT). PEComas are more commonly encountered intra-abdominally, although there are now increasing reports of PEComas occurring cutaneously. We present two further rare cases of PEComas occurring in soft tissues and highlight the fact that most reported PEComas of soft tissues to date, including ours, seem to have a tendency to occur in the lower limb.[1–4]

The first case was a 15-year-old female with a painless, gradually enlarging lump on her thigh for over 2 years. Past medical history was unremarkable apart from asthma, and there was no history of tuberous sclerosis. The lesion was presumed to be a sebaceous cyst and excision of the lesion was performed with later wider excision. A magnetic resonance imaging scan was negative and follow-up at 1 year showed no recurrence.

The second case was a 38-year-old female who presented similarly with a painless lump in the posterior thigh enlarging over a period of 18 months. There was no past history of note and she too underwent excisional biopsy followed by wider excision.

In the first case, sections showed tumour extending from the dermis into the subcutaneous fat with a very organoid pattern and well-circumscribed nests. There was prominent vascular stroma surrounding these nests. Cells had central oval nuclei and abundant clear to finely granular cytoplasm (Figure 1). There was only mild variation in nuclear size and shape, which were eosinophilic. Periodic acid–Schiff (PAS) revealed abundant granular material, which disappeared with diastase PAS. A few small irregular fragments remained, but true rod-like crystals were not identified. The impression initially was that of an alveolar soft part tumour, but G-band analysis of monolayer tumour cultures showed a normal female karyotype with no evidence of t(X:17) translocation on fluorescence *in situ* hybridization, studies typical of alveolar soft part tumours. Immunohistochemistry showed cells which were positive for HMB-45, CD63, chromogranin, CD68 and Melan-A, but negative for S100, desmin, smooth muscle actin (SMA), cytokeratins, epithelial membrane antigen (EMA), CD56 and MyoDi.

In the second case, histological examination showed tumour extending from the dermis into the underlying subcutaneous fat. It was composed of large cells arranged in nests and poorly defined fascicles. The cells had central rounded nuclei and abundant pale to clear cytoplasm, which was often vacuolated. In some areas the cells appeared spindled with more eosinophilic cytoplasm and more oval nuclei. There was mild variation in nuclear size and shape. There was no tumour necrosis and only one mitosis per 20 high-power fields. The tumour was positive for HMB45 (Figure 2), Melan-A and CD68. There was weak positivity for SMA. The tumour was negative for MNF116, S100, CD34, AE1/AE3, desmin and EMA.

The apparent tendency for PEComas to occur in the lower limb when affecting skin is curious. There are too few documented cases to draw any real conclusions from this trend. It may be explained simply by the fact