SYNOVIAL LINING MACROPHAGES MEDIATE OSTEOPHYTE FORMATION DURING EXPERIMENTAL OSTEOARTHRITIS

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INTRODUCTION.

During osteoarthritis (OA) many pathological changes are observed in the diseased joints. Those changes include loss of articular cartilage and sclerosis of subchondral bone, but also the synovium is involved. Inflammation of the synovium, synovitis, is demonstrated and formation of osteophytes (new formation of bone and cartilage) at the junction of bone and synovium is a common feature in OA. Although certain growth factors like transforming growth factor (TGF)-β and bone morphogenetic protein (BMP)-2 have been implicated in osteophyte formation, the mechanisms via which this occurs have to be elucidated.

In experimental models for arthritis osteophyte formation is also found, which suggests that synovitis can induce formation of osteophytes. Macrophages from the synovial lining have been shown to determine inflammation and cartilage damage in experimental arthritis. Both are ameliorated, when synovial macrophages are selectively depleted from the synovial lining layer during several models of experimental arthritis. In addition, this selective lining depletion prevented osteophyte formation.

This leads to the question whether macrophages are involved in osteophyte formation that occurs during experimental OA and whether synovial inflammation plays a role in this.

METHODS.

As an animal model for OA collagenase induced osteoarthritis was used. Two groups of 16 C57Bl/6 mice were injected twice with collagenase in the right knee joint on alternate days. One group had been injected intra-articularly with macrophage depleting clodronate liposomes 1 week prior to collagenase injection. Right knee joints were dissected 7 days and 14 days after the first collagenase injection and subsequently processed for histology. Histological sections were stained using hematoxylin/eosin (HE) and Safranin-O staining. Subsequently osteophyte formation, fibrosis and inflammation were determined. Osteophyte formation was measured using an image analyzer to determine the osteophyte area on three sections per knee joint. Also activation of macrophages was detected using MRP8/14 immunostaining. In addition, the role of macrophages in TGFβ-induced osteophyte formation was studied by depleting macrophages from the synovial lining prior to triple intra-articular injection with 200 ng TGFβ.

RESULTS.

At day 7 after collagenase injection MRP8/14 staining showed a distinctly positive lining layer and also some macrophages in deeper layers stained positive. This indicates that in the early phase of osteoarthritis macrophages are activated.

Osteophyte formation at day 7 was already substantial (Figure 1A), whereas at day 14 large osteophytes were found of which some were already mineralized (Figure 1C). When macrophages were depleted from the lining prior to induction of osteoarthritis, osteophyte formation was largely prevented. At day 7 the mean osteophyte size was 76±17 μm^2 in control joints and 12±6 μm^2 ($p<0.01$) in macrophage depleted joints, whereas at day 14 the mean size was respectively 305±70 and 104±17 μm^2 ($p<0.03$).

When fibrosis as well as inflammation was determined in the synovium, also differences were found between macrophage depleted and non-depleted joints. Fibrosis decreased from 1.0±0.3 to 0.5±0.2 at day 7, and from 1.6±0.3 to 0.6±0.3 at day 14, in macrophage depleted compared to non-depleted joints. Although only a very low level of synovitis was found at day 7 and 14, a significant decrease was found in macrophage depleted joints (decrease 50%). This indicates that macrophages play a role during osteophyte formation, fibrosis and synovitis.

To further investigate whether macrophages are involved in TGFβ induced osteophyte formation, TGFβ was injected into knee joints of control mice and of mice from which synovial macrophages were depleted. Control mice showed substantial osteophyte formation whereas the depleted joints showed hardly any chondro- or osteogenic processes (figure 2).

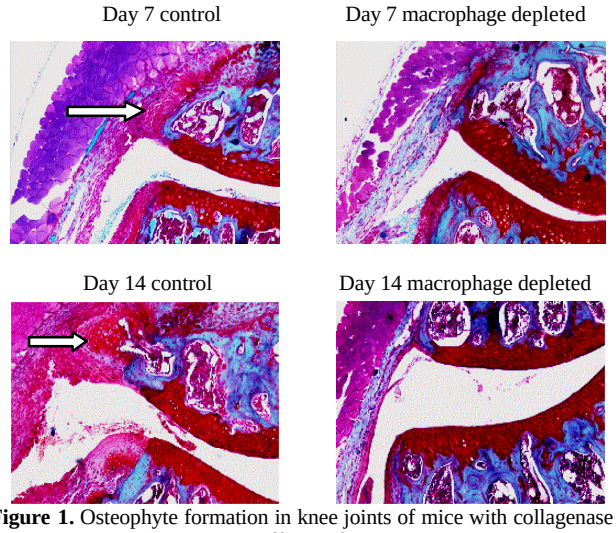

Figure 1. Osteophyte formation in knee joints of mice with collagenase induced osteoarthritis. The effect of macrophage depletion was determined after 7 and 14 days.

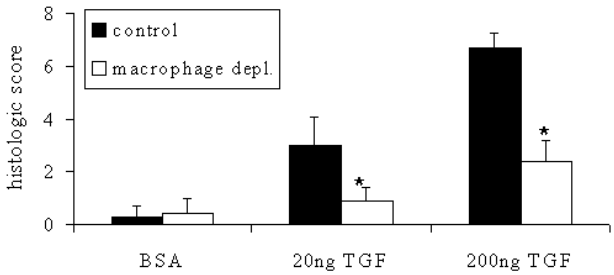

Figure 2. Effect of lining depletion on TGFβ-induced osteophyte formation (black bars: controls, open bars: after macrophage depletion).

DISCUSSION.

In this study we clearly demonstrate an important role of synovial lining macrophages in osteophyte formation and fibrosis. It has already been suggested that synovitis might take part in OA pathology. Indeed in our study we demonstrated that specific depletion of synovial macrophages caused amelioration of the already low level of synovitis. Apart from a possible role for synovitis in osteophyte formation, also TGFβ is important in this. We demonstrate that lining macrophages are involved in TGFβ-induced osteophyte formation. From literature it is known that TGFβ has a direct chondro- or osteogenic effect on periosteal cells. Our data show that stimulation of macrophages with TGFβ in addition leads to generation of factors that facilitate this osteophyte formation.

Therefore, two separate mechanisms may underlie this important role for macrophages in osteoarthritic changes in the joint. 1: Macrophages are involved in perpetuation of synovitis. 2: Macrophages stimulated by TGFβ produce mediators involved in induction of chondrogenesis. Possibly synovitis contributes to production of TGFβ in the synovium, which in turn causes macrophages to produce other growth factors. This role for macrophages in osteophyte formation is a new finding, however, during healing of bone fractures, macrophages are among the first cell types to arrive at the site of injury and are thought to play an active role in induction of chondrogenesis. This study identifies the macrophage as a key player in the generation of osteophytes and induction of fibrosis during OA. Whether macrophages are involved in other OA related pathology, like cartilage erosion, is under investigation.