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**HISTOPATHOLOGY**

**DISCUSS THE INVOLVEMENT OF T AND B LYMPHOCYTES IN THE PATHOGENESIS AND PROGRESSION OF OSTEOMYELIIS AND OSTEOARTHRITIS**

**THE ROLE OF T CELLS IN THE PATHOGENESIS OF OSTEOARTHRITIS (OA)**

Osteoarthritis is the degeneration of joint cartilage and the underlying bone, mostly common from middle age onward. T cell is a type of lymphocyte that develops in the thymus gland and plays a role in the immune response.

Enzyme-linked immunosorbent assay analysis revealed higher levels of sCD4 not only in the peripheral blood but also in the synovial fluid of patients with OA, compared with age-matched healthy controls, which suggests that Th cells in the synovial fluid are involved in the pathogenesis of OA (Symons *et al* 1991). When stimulated with PMA and ionomycin, mononuclear cells from the synovial fluid of OA patients showed a high expression of CD4 and CD8 markers (Dolganiuc *et al* 1999). These compelling results suggested that T cells in the synovial fluid are associated with the pathogenesis of OA. The percentage of T cells in the synovial fluid of OA patients was found to be significantly higher than that in their peripheral blood (van de Putte *et al* 1975) and T cells in the synovial fluid of OA patients expressed class II HLA (an indicator of activated T cells) (Haynes *et al* 2002). The percentages of CD4+ and CD8+ cells in the synovial fluid of OA patients were even similar to those found in RA patients (Hussein *et al* 2008). T cells are the major constituents of synovial infiltrates in the membranes of OA patients, and both CD4+ T cells and CD8+ T cells have been found within synovial aggregates (Haynes *et al* 2002). The synovial tissue extracted from OA patients displayed perivascular CD3+ T cell infiltration at an early stage (Nakamura *et al* 1999). Similarly, using immunohistochemical analysis, CD3+, CD4+, and CD8+ T cells were detected predominantly in the sub lining layer and more limitedly in the deep layer of the synovium of patients with OA, whereas the presence of CD4+ T cells in the synovial sub lining layer was detected more strongly in OA patients than in normal people (Ishii *et al* 2002). CD4+ T cells were found to be predominant among the T-cell infiltrates in the synovial tissue, and the number of CD4+ T cells was higher in the synovial sub lining layer of patients with OA than in that of normal people. The medial synovium of patients with knee OA has been shown to contain more CD4+ T cells than the lateral synovium. Synovial aggregates from OA patients express CD80, an inducible costimulatory ligand involved in T-cell activation (Nakamura *et al* 1998), suggesting that synovial aggregates in OA patients are areas of antigen recognition and T-cell activation. Similarly, research involving 30 patients with OA found CD3+ T cell aggregates in the synovial membrane in 65% of the patients, and the activation antigens CD69, CD25, CD38, CD43, CD45RO, and HLA class II were also found in the synovial membrane (Nakamura *et al* 1998). Also HLA-antigen D-related (DR)-expressing T cells were found in the synovial membranes of OA patients using immunohistochemical analysis, although to a lesser degree than in RA patients (sakkas *et al* 1998). The conclusion that activated T cells are aggregated in the synovial membranes of OA patients was further supported by the discovery that virtually all T cells in OA joints express activation markers, such as HLA-DR and CD69 (40). OA patients older than 75 have higher percentages of CD3+, CD4+, and CD8+ cells in their synovial membranes than OA patients younger than 75 (Pawlowska *et al* 2009). This suggests that age is among the risk factors for OA. Significant abnormalities in the T-cell profile have been found in the peripheral blood, synovial fluid, and synovial membranes of OA patients.

**THE ROLE OF B CELLS IN THE PATHOGENESIS OF OSTEOARTHRITIS**

Ageing is a complex phenomenon. It affects cells and tissues, diminishes homeostasis and increases vulnerability. Many pathways have been shown to be involved in ageing and age-related diseases, including osteoarthritis (OA). In elders, infectious diseases are the primary cause of death, underpinning the role of the immune system. The acquired immune responses decline with age (increased susceptibility to infection, poor responses to vaccination, higher prevalence of cancers). In addition, there is intrinsic difficulty in dealing with common pathogens and a disproportionate inflammatory response. This decline is accompanied by an increase in auto-reactivity (generation of autoantibodies) and chronic low-grade inflammation, which acts as predictor of mortality. Inflammatory response is so prevalent in driving tissue damage associated with age-related diseases that the term "Inflammageing" has been coined to explain the underlining inflammatory changes common to most age-associated diseases. OA is a degenerative joint disease whose prevalence increases with age, and includes a group of pathologies involving structural degeneration of the joint resulting in pain and disability. There is evidence of OA in all individuals over the age of 60 but symptomatic OA, (disease that requires medical treatment), occurs in only 15%. The reasons why OA only becomes symptomatic in some people remain unexplained. Recent magnetic resonance imaging studies have highlighted a very high frequency of pathology involving cartilage, bone and synovium (soft tissue lining of the joints), with relevance in structure-pain associations. Inflammation of the synovium (synovitis) is a well-recognized feature of OA, notably with an important role for interleukin-1beta (IL-1β). Many of the age-related defects of the immune system highlighted above are involved in the pathogenesis of OA. Furthermore, mechanical forces which produce cartilage damage breakdown products have been proposed as a source of immune stimulation, promoting persistent low-grade inflammation. Antigen-driven stimuli using collagen breakdown products as neo-antigens were suggested to lead to specific T/B-cell responses. Innate immune responses to calcium crystal deposition were shown to initiate IL-1β production. The interactions between the musculoskeletal and the immune system are important sources of divergence between healthy people and patients with OA. We postulate that they can be used to better understand the pathology of OA by distinguishing ageing specific changes from those that are OA specific. We recently showed that the immune cell composition of the blood of OA patients is quite divergent from that of aged-matched controls notably with major changes in CD4/CD8 T-cells, loss of regulatory T-cells and alteration in the T to B-cell ratio. Synovitis has been proposed as niche for B-cell maturation and the production of auto-antibodies in OA. However, B-cell infiltration in OA is quite independent of T-cells and the presence of germinal centre like structures is rarely observed. The IgM repertoire is quite broadly developed (targeting many auto-antigens in health even at a younger age), however maturation of IgG is achieved with the help of T-cells. In ageing (and OA) T-cell help is defective and alternative signals can be used to mature B-cells. These include signals from the innate immune system, such as those provided by the activation of TLR on B-cells which result in the expression of the XBP-1 transcription factor, an essential regulator of B-cell maturation. The events leading to B-cell maturation and isotype switching are known and steps are reproducible in vitro. B-cell cultures will be used to establish the role of TLR activation on B-cell maturation and effect on the activation of XBP-1. Gene expression profiling will address the pathways implicated in this alternative maturation process including XBP-1 and genes of the B-cell receptor and immunoglobulin rearrangement pathways. These profiles will be compared to those obtained from B-cell cultures stimulated with T-cell help signals (CD40::CD40L). Selected genes will then be tested in B-cells purified from the blood from healthy controls and OA patients and from OA synovial tissue to assess the mean by which B-cells mature and produce IgGs (i.e. T-cell dependent or independent pathways).

**THE ROLE OF T-CELL IN THE PATHOGENESIS AND PROGRESSION OF OSTEOMELYTIES**

Chronic bone infection is associated with bone resorption. From animal studies, CD3/CD28-activated T cells are known to enhance osteoclastogenesis and bone resorption. Because CD28 is expressed constitutively on T cells and its expression is down-regulated by chronic exposure to the inflammatory environment, we characterized co-stimulatory molecule expression on T cells from chronically infected patients. BI, or osteomyelitis, is an inflammatory process accompanied by bone destruction. T lymphocytes have been recognized as key regulators of osteoclast (OC) and osteoblast (OB) formation, lifespan and activity (Horwood *et al* 1999). OC shows some characteristics of antigen-presenting cells (APC) in experimental models of inflammatory diseases (Li H *et al 2010*), which imply an interaction of OC with T cells. It has been demonstrated that activated T cells exert their effect via membrane-bound and secretory receptor activator of nuclear factor kappa B ligand (RANKL) (Wyzga N *et al* 2004). In one in-vitro study it was found that CD4+ T cells can support osteoclastogenesis in the presence of macrophage colony-stimulating factor (M-CSF) alone, and the addition of RANKL led to increased bone resorption (Kong *et al* 1999). Conversely, CD8+ T cells did not support OC differentiation, but rather inhibited OC differentiation/activation induced by RANKL (Buchwald *et al* 2012). CD3/CD28 stimulation was used to study lymphocyte–OC interactions (Horwood *et al* 1999). However, co-stimulatory molecules, CD28, CD80, CD86, cytotoxic T-lymphocyte antigen-4 (CTLA-4)/CD152, CD40 and CD40L showed major alterations of expression in bacterial infection, which may be responsible for modulating cellular interactions (Cacere *et al* 2008). Analysis of synovium of patients with chronic septic arthritis showed dramatic T cell proliferation. This proliferation of T cells suggests the occurrence of some major cellular interactions (Pessler *et al* 2008). Apoptosis might be related to regulatory T cells which are specialized to limit the magnitude of T cell effector responses (Singh *et al* 2010). Infected bones are characterized by higher T cell activation but fewer proliferations compared to non-infected bones, regardless of the T cell subset considered. It was shown that in mouse, bone marrow is highly enriched for the activated/memory phenotype (Price *et* Cerny 1999). Also, the population of naive CD4 T cells in bone marrow was 44%, which decreased after having microbial antigen exposure with subsequent increase of memory phenotype. T cells at inflammatory sites undergo an activation process, during which they lose the adhesion protein CD62L, facilitating the cell transmigration process (Sallusto *et al* 1999). In a mouse model of alveolar bone resorption induced by Porphyromonas gingival is, only CD4 T cells appeared to be involved in the osteoclastogenic process (Baker *et al* 1999). In-vitro osteoclastogenesis by activated T cells through RANKL has also been shown using murine CD4 T cells or human PBMC-derived T cells (Horwood *et al* 1999). In implant-associated post-traumatic osteomyelitis it has been shown that T cells down-regulate the expression of CD62L when infiltrating the inflamed site (Wagner *et al* 2006). Thus others indicate T cell activation and differentiation in infected bone tissue. Decreased T cell proliferation in infected bone tissue might be the result of tissue infiltration by Tregs. Tregs are believed to induce anergy and ultimately lead to T cell apoptosis, which may be phagocytized rapidly, as seen in physiological conditions (Belkaid *et* Tarbell 2009). The CD28 molecule on T cells interacts with B7 molecules on APC for optimal expansion of the T cell response (Gray *et al* 2002). CD28 is expressed constitutively on T cells, being largely increased early in cellular activation, while CTLA-4 is expressed on the cell surface only after activation (Wang *et* Chen 2004). The absence of CD28 expression is commonly observed on the CD8 T cell subset with advance in age (Filaci *et al* 2004). Also, increase of CD28−CD8+ T cells has been reported in chronic inflammation, in both experimental and human diseases. There’s an up-regulation of the CD40/CD40L pathway on both T cell subsets, suggesting a long process of T cell activation. CD40–CD40L interaction is required for both a prolonged T cell response against pathogens and antibody production, as shown in mouse models challenged with different kinds of pathogens (Elgueta *et al* 2009). Bone tissue in humans appears to be a site of T cell activation and proliferation, the latter being decreased in chronic bacterial infections. Bone infections are associated with a high percentage of CD28− CD4 T cells exhibiting a cytotoxic phenotype as well as an up-regulated CD40/CD40L pathway on T cells. As activated T cells are already known to express RANKL, which is a crucial factor for osteoclastogenesis, our results suggest a creative line of work on the mechanisms of bone resorption in human BI. Moreover, we assume that biotherapies targeting co-stimulatory molecules might have significant but variable effects on bone resorption, depending on their membrane expression.

**THE ROLE OF B-CELL IN THE PATHOGENESIS AND PROGRESSION OF OSTEOMELYTIES**

Bone malignancies can present with signs and symptoms mimicking osteomyelitis. Hence there is possibility of delayed diagnosis of malignancy or it may be initially misdiagnosed as osteomyelitis, which can adversely affect the outcome. osteomyelitis can be said to be where there is any deviation from its typical natural history, progression or unexpected histological change in the bone. Primary bone lymphoma constitutes 3% of primary bone tumor and 5% of extranodal lymphomas. Males are affected more commonly than females (Mika *et al* 2012). Although primary bone lymphoma is uncommon, all ages may be affected. Common sites of involvement include femur, humerus, tibia, spine, pelvis, sternum, ribs, and bones of the skull and face in the decreasing order. The exact etiology of primary bone lymphoma is unknown, in some cases; immunosuppression or viral agents are attributed. Bone pain is the most common presenting feature of bone lymphoma. As of now, there is no report of lymphomas being developed secondarily in chronic inflammatory conditions. Regional lymph nodes may be involved in some patients although this occurs more commonly in cases of bone involvement in patients with systemic lymphoma. Malignant lesions may be misdiagnosed as a rise or accompany osteomyelitis. There are only a very few such case reports in the literature regarding primary bone lymphomas (Ganapathi *et ql* 2001). Histologically, there are many types of inflammatory cells in lymphoma, the most common being the T-lymphocyte infiltrate, which will obscure the lymphoma cells and gives an incorrect diagnosis of osteomyelitis. Lymphoma cells, although are, may also be spindle shaped and can be arranged in a storiform pattern which may masquerade as sarcoma. The first-line marker for differentiating between lymphomas (CD45+) and poorly differentiated nonhemopoietic tumors (CD45−) is the monoclonal antibodies directed against the leukocyte common antigen (CD45) (Inaba *et al* 2008). Most common type of malignant lymphoma of bone is B-cell neoplasms. Lymphoid origin can be confirmed by CD45 (LCA) stain (Inaba *et al* 2008). Most B-cell neoplasms show the presence of CD20 on the cells and are absent on otherwise similar appearing T-cellneoplasms. The B-cell lineage can be by CD20 stain (Inaba *et al* 2008). It is very useful in diagnosing conditions such as B-cell lymphomas; however, the presence or absence of CD20 in such tumors is not relevant to prognosis (Cooper *et* Leong2003). CD99 was negative which is specific of Ewing sarcoma, and both CD45 and CD20 were positive to prove it is a B-cell lymphoma without any confusion. Delays in establishing the diagnosis have serious effects on the prognosis. The disease stage being often considered as the most important prognostic indicator (Ostrowski *et al* 1986). Hence, earliest diagnosis prior to leukemic spread or metastasis is highly essential. In any patient with osteomyelitis like picture and bone pain refractory to medical treatment, the detailed diagnostic workup should be done, and unusual neoplasms like primary lymphoma of bone should be considered in the diagnostic armamentarium. Commonly, lymphoma is expected to have a progressively worsening course, but one of the types of primary B-cell lymphoma has an indolent (slow-growing) course or has clinical regression. We believe our case might be of this variety.

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