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ASSIGNMENT QUESTION: Discuss the involvement of T- and B-lymphocytes in the pathogenesis and progression of osteomyelitis and osteoarthritis.

OSTEOMYELITIS

Osteomyelitis is inflammation of the bone caused by an infecting organism. Although bone is normally resistant to bacterial colonization, events such as trauma, surgery, the presence of foreign bodies, or the placement of prostheses may disrupt bony integrity and lead to the onset of bone infection. Osteomyelitis can also result from hematogenous spread after bacteremia. When prosthetic joints are associated with infection, microorganisms typically grow in biofilm, which protects bacteria from antimicrobial treatment and the host immune response. (Stephen, 2018).

The severity of the long bone osteomyelitis is staged depending upon the infection's particular features, including its etiology, pathogenesis, extent of bone involvement, duration, and host factors particular to the individual patient (infant, child, adult, or immunocompromised). Osteomyelitis can be classified by duration (acute or chronic), pathogenesis (trauma, contiguous spread, hematogenous, surgical), site, extent, or type of patient. (Jason *et.al*., 2009).

PATHOGENESIS OF OSTEOMYELITIS

Osteomyelitis may be caused from hematogenous spread, direct inoculation of microorganisms into bone, or from a contiguous focus of infection. A trivial skin infection may be the source of bacteremia or it may emerge as the result of a more serious infection such as acute or subacute bacterial endocarditis. Hematogenous osteomyelitis usually involves the metaphysis of long bones in children or the vertebral bodies in adults. With hematogenous osteomyelitis, the joint is usually spared from infection in children, unless the metaphysis is intracapsular, as is found at the proximal radius, humerus, or femur (Dhal *et al*., 1998). The most common causes of direct inoculation

osteomyelitis are penetrating injuries and surgical contamination. Contiguous focus osteomyelitis commonly occurs in patients with severe vascular disease (Jason *et.al*., 2009).

Host factors are primarily involved with containment of the infection once it has been introduced adjacent to or into the bone, but on occasion, specific host factors may predispose individuals to develop osteomyelitis. Host deficiencies that favor bacteremia thus favor the development of hematogenous osteomyelitis. Host deficiencies involved with direct inoculation of organisms and/or contiguous spread of infection from an adjacent area of soft tissue infection are primarily involved with the lack of containment of the initial infection. patients with sickle cell anemia, chronic granulomatous disease, and diabetes mellitus have a notable susceptibility to acute skeletal infections (Coles *et.al.,* 1991).

Acute osteomyelitis presents as a suppurative infection accompanied by edema, vascular congestion, and small vessel thrombosis. In early acute disease, the vascular supply to the bone is decreased by infection extending into the surrounding soft tissue. Large areas of dead bone (sequestra) may be formed when the medullary and periosteal blood supplies are compromised (Emslie *et.al*., 1983). Acute osteomyelitis can be arrested before dead bone develops if treated promptly and aggressively with antibiotics and surgery (if necessary). In an established infection, fibrous tissue and chronic inflammatory cells form around the granulation tissue and dead bone. After the infection is contained, there is a decrease in the vascular supply to it, inhibiting an effective inflammatory response. Chronic osteomyelitis is the result of the coexistence of infected, nonviable tissues and an ineffective host response (Ciampolini and Harding, 2000).

Bacteria have been shown to persist within glycocalyx-enclosed microcolonies adherent to the bone and to prosthetic devices in cases of osteomyelitis (Gristina *et.al*., 1985). Biofilms are typically composed of cells embedded in a highly hydrated polysaccharide matrix with nucleic acids and proteins throughout. These biofilms are associated with the refractory nature of chronic infections such as osteomyelitis (Anwar *et.al*., 1990). Concentrations of antimicrobial agents required for the eradication of bacteria in biofilms are more than 50 to 1000 times higher than those needed for killing of the free-floating planktonic cells. Usually, that level of antibiotics is impossible to achieve because of patient toxicity. In the bones, this is further complicated by questionable penetration of antibiotics into infected and ischemic areas leading to subpotent antibiotic concentrations (Anwar *et.al*.,1990). The reason for the reduced ability of antimicrobial agents to eradicate these infections is due to the reduced antibiotic penetration and the very slow growth rate and differential upregulation of stress response genes by cells within the biofilm.

Pathologic features of chronic osteomyelitis are the presence of necrotic bone, the formation of new bone, and the exudation of polymorphonuclear leukocytes joined by large numbers of lymphocytes, histiocytes, and, occasionally, plasma cells. New bone forms from the surviving fragments of periosteum and endosteum in the region of the infection. An encasing sheath of live bone, an involucrum, surrounds the dead bone under the periosteum. The involucrum is irregular and is often perforated by openings through which purulence may track into the surrounding soft tissues and eventually drain to the skin surfaces, forming a chronic sinus. The involucrum may gradually increase in density and thickness to form part or all of a new diaphysis. New bone increases in amount and density for weeks or months, according to the size of the bone and extent and duration of infection. (Jason H. *et.al*., 2009). Endosteal new bone may proliferate and obstruct the medullary canal. After host defense or operative removal of the sequestrum, the remaining

cavity may fill with new bone, especially in children. However, in adults, the cavity may persist or the space may be filled with fibrous tissue, which may connect with the skin surface via a sinus tract (Ciampolini and Harding, 2000).

Involvement of T and B lymphocytes in osteomyelitis

Lymphocytes get activated during inflammation as an immune response.

Activated T cells may undermine bone homeostasis and stimulate bone destruction under pathological conditions such as estrogen deficiency (Cenci S. *et.al*., 2003) and in inflammatory conditions (Kong YY *et.al*., 1999) (kawai T. *et.al*., 2006) as they become a significant source of receptor activator of NFκB ligand (RANKL) and TNFα (Cenci S. *et.al*., 2000).

Interestingly, T cells are also capable of mediating antiosteoclastogenic signals, as depletion of CD4+ and CD8+ T lymphocytes in mice in vivo enhances vitamin D3–stimulated osteoclasts (OC) formation in vitro by a mechanism involving decreased osteoprotegerin (OPG) production (Lee SK *et.al.*, 2000). The source of OPG and the mechanism of its regulation are not fully elucidated. In addition, CD8+ T cells have been reported to be antiosteoclastogenic (John V. *et.al*., 1996). Interestingly, although bone mineral/marrow density (BMD) is normal or elevated in very young C57BL/6 T-cell–deficient nude mice, BMD decreases as the mice age. This suggests that T cells may play a protective role in postembryonic basal bone modeling; however, the mechanisms of OC repression by T cells in vivo remain to be elucidated (Yan Li. *Et.al*., 2007).

B cells and B-cell–derived plasma cells in multiple myeloma have been reported to have the potential to support osteoclastogenesis, possibly via direct expression of RANKL or as an indirect consequence of IL-7 secretion, a potent stimulator of bone resorption in vivo.

B lymphopoiesis is stimulated during estrogen deficiency while estrogen treatment down-regulates B lymphopoiesis but up-regulates immunoglobulin production. B-lineage cells have consequently been suggested to play a role in ovariectomy-induced bone loss. B-cell precursors are capable of differentiating into OCs in vitro, suggesting that estrogen deficiency may expand the reservoir of OC precursors. (Yan Li. *Et.al*., 2007).

Peripheral blood B cells inhibit OC formation in a human in vitro model of osteoclastogenesis, in part through secretion of TGFβ, a cytokine that induces apoptosis of OCs and that is reported to stimulate OPG production (Miles RR *et.al*., 2001). Depletion of B cells in vivo also aggravates bone loss in an animal model of periodontitis, suggesting that B cells may act to limit bone resorption under certain pathological conditions (Klausen B *et.al.,* 1989).

Furthermore, B-cell to T-cell crosstalk may regulate B-cell production of bone-active cytokines, because B cells suppress osteoclastogenesis when activated by Th1 cytokines while promoting osteoclastogenesis when stimulated with Th2 cytokines. In vitro ligation of the costimulatory molecule CD40 on human tonsil-derived B cells with an activating antibody is reported to stimulate B-cell OPG production.(Yun TJ *et.al*., 1998) Physiologically CD40 interacts with its cognate ligand, CD40 ligand (CD40L), a molecule expressed on activated T cells during antigen presentation by antigen-presenting cells (APCs) such as B cells, macrophages, and dendritic cells

and acts in priming of naive CD8+ cells. Whether T-cell to B-cell crosstalk contributes to regulate basal bone turnover in vivo is presently unknown. (Yan Li. *Et.al*., 2007).

Under pathological conditions, both T and B cells cooperate to play a critical role in limiting basal bone resorption in vivo. This protective effect is centered on a mechanism involving the production of OPG by B-lineage cells, and augmented by T cells, via CD40/CD40L costimulation.

OSTEOARTHRITIS (OA)

Osteoarthritis is the common type of arthritis. Osteoarthritis (OA) is a chronic disease which results from damage to articular cartilage induced by a complex interplay of genetic, metabolic, biochemical, and biomechanical factors followed by activation of inflammatory response involving the interaction of cartilage, subchondral bone, and synovium (lancet, 1997).

Many factors contribute to an increased risk of Osteoarthritis. This include obesity, genetics, aging and trauma to the joint. In most patients without a strong genetic predisposition, OA can be as a result of damage to the joint tissue by physical forces as a single event of trauma or by repeated microtrauma due to altered mechanical loading of the joint (Brandt, 2009). Chondrocytes respond to the physical injury by stopping the production of anabolic factors and by releasing more catabolic enzymes such as MMPs, which results in further damage to the cartilage and this further leads to the release of matrix components, which elicit inflammatory mechanisms (Jasin, 1988). Involvement of an immune response, both innate and adaptive, in OA is now widely accepted based on the following evidence:

1. An inflammatory synovium/synovitis has been linked to increased cartilage damage and pain in recent epidemiological studies on large number of OA patients.
2. Infiltrates of immune cells including T-cells, B-cells and macrophages have been detected in synovial tissue of OA patients.
3. Immunoglobulins and immune complexes against cartilage components are detected in cartilage, synoium and plasma in OA patients.
4. Key role of complement activation in OA synovium has been identified.

Involvement of T lymphocytes in Osteoarthritis (OA)

T lymphocyte, type of leukocyte (white blood cell) that is an essential part of the immune system.

T cell plays roles in the osteoarthritis pathogenesis. Generally, osteoarthritis is said to be a non-inflammatory disease but studies have shown inflammation in the synovial membrane indicating the features of T cell immune response.

1. CD3+ T cells infiltrate the synovial membrane of patients with OA; presence of mononuclear cell (MNC) infiltrates consisting of T cells and macrophages in the synovial membrane of >50% of patients with OA. MNC infiltrates may be diffuse or perivascular nodular. Angiocentric infiltrates composed primarily of CD3+ T cells in the synovial membrane of patients with OA.
2. T cells infiltrating the synovial membrane of patients with OA express early, intermediate, and late activation antigens: T cells infiltrating the synovial membrane express early activation antigens (CD69), intermediate activation antigens (CD25, CD38), and late activation antigens (CD45RO, HLA class II) (Sakkas Li et.al., 1998). These activation

antigens were expressed on T cells and other MNCs infiltrating the synovial membrane of both patients with OA and patients with RA, although their proportions were significantly higher in patients with RA than in those with OA (Sakkas Li *et.al*., 1998). Although CD45RO+ T cells may extravasate from peripheral blood, the expression of CD69, an early activation antigen, suggests that activation occurs in situ, in the synovial membrane. CD38 and the CD43, which are detected in the synovial membrane of patients with OA (Sakkas Li *et.al*., 1998), mediate adhesion to vascular endothelium and binding to intercellular adhesion molecule 1 (ICAM-1), respectively. Leukocytes and endothelial adhesion molecules are also expressed in the synovial membrane of patients with OA. B cells are also activated in patients with OA.

3. HLA association of OA: Several studies have demonstrated associations of OA with HLA class I and HLA class II alleles. This HLA class II association of OA further supports the concept that OA, at least in certain patients, may be a trimolecular-complex (T cell receptor [TCR]/antigen/HLA) disease. Interestingly, the normally HLA–DR–negative chondrocytes become positive in OA, suggesting that they may function as antigen-presenting cells (APCs). T cells derived from the peripheral blood or synovial fluid of patients with OA responded to membrane preparations of autologous chondrocytes and fibroblasts by proliferation. These T cell responses are monocyte dependent, suggesting an antigen-specific immune response (Kalden JR et.al., 1990).
4. T cell cytokines are produced in the synovial membrane of patients with OA: interleukin-2 (IL-2), interferon-γ (IFNγ), and IL-10 transcripts in the synovial membrane of 50% of patients with OA. IL-4 or IL-5 transcripts were not detected by polymerase chain reaction

(PCR) amplification in the synovial membrane of patients with OA, suggesting the presence of a Th1 cytokine pattern in the synovial membrane of patients with OA.

Quantitative PCR analysis using MIMIC demonstrated that IFNγ transcript levels in OA, when normalized for T cell equivalents. This means that T cells infiltrating the synovial membrane of patients with OA are as active as those infiltrating the synovial membrane of patients with RA, although they are present in lower numbers in OA . IFNγ protein was detected by immunohistochemistry in the synovial membrane of most patients with OA. Th1-type cytokine transcripts were also found in MNCs from the synovial fluid of patients with OA. Both IFNγ protein and IL-4 protein were detected in the synovial fluid of patients with OA (sakkas L. and Chris D, 2007).

Because IL-12 is a major inducer of Th1 cytokines, IL-12 was detected in the synovial membranes of OA patients, at both the messenger RNA level (IL-12 p40) and the protein level (IL-12 p70) (sakkas L et.al., 1998). IL-12, which is produced by macrophages during phagocytosis, even of inert material, may drive the cytokine pattern in the OA synovial membrane toward the Th1 pattern (sakkas L *et.al*., 1998). In addition to IL-12, other molecules may participate in the Th1 cell response in OA, including chemokines such as IL-8 and macrophage inflammatory protein 1α (MIP-1α). T cells producing Th1 cytokines express CCR5 on the cell surface. CCR5 is a receptor for MIP-1α, a T cell chemoattractant that is up-regulated in the synovial fluid of patients with OA (Koch AE *et.al*., 1995). Th1 cells may be driven into the synovial membrane of patients with OA by inciting antigens and/or IL-12 or chemokines. IL-10 transcripts have been observed in the synovial membrane of nearly all OA patients examined, often in addition to IFNγ and IL-2 transcripts (sakkas L *et.al*., 1998). IL-10 has been classified as an antiinflammatory Th2

cytokine in mice (Joosten LA *et.al*., 1994). IL-10 in humans cooperates with IL-4 to inhibit the production of proinflammatory cytokines by adherent rheumatoid synovial cells (Taki H *et.al*., 1995). However, IL-10 in humans is produced by both monocytes and Th1 cells (Anderson *et.al*., 1996). In conclusion, proinflammatory Th1 cytokines (such as IFNγ and IL-2) and IL-10 are expressed in the synovium of patients with OA.

Peripheral blood mononuclear cells (PBMCs) from patients with OA have been shown to express levels of CCR1, CCR3, CCR5, CCR6, and CCR7 chemokines comparable with the levels expressed by PBMCs from patients with rheumatoid arthritis (RA). Serum levels of the activation-induced T cell–derived chemokine-related cytokine lymphotactin, which is a lymphocyte chemoattractant, were similar in patients with OA and those with RA (Blaschke *et.al*., 2003).

5. Autoantibody responses in OA: The presence of autoantibodies to arthritis-related antigens during the early stage of knee OA suggests that a specific immune response may be responsible for the initial degradation of cartilage in OA (Du H et.al., 2004).
6. T cells infiltrating the synovial membrane of patients with OA contain oligoclonal populations of T lymphocytes.
7. T cells from the peripheral blood and synovial fluid of patients with OA exhibited strong proliferative responses to preparations of autologous chondrocyte membranes and autologous fibroblast membranes but not to epithelial cell membranes (Kalden JR *et.al*., 1990). In RA, however, proliferative responses only to preparations of autologous chondrocyte membranes and not to autologous fibroblast or epithelial cell membranes have been reported (Kalden JR *et.al*., 1990).

Studies at the clonal level revealed high precursor frequencies in T cells responding to chondrocyte membranes in the peripheral blood of patients with OA. These T cell responses were strongly monocyte dependent and exhibited the characteristics of a specific antigen–driven process (Kalden JR *et.al*., 1990).

The role of cartilage oligomeric matrix protein (COMP) as an autoantigen capable of eliciting T cell responses in patients with OA should be studied. COMP is derived primarily from the cartilage and the synovial membrane of patients with OA (Clark AG *et.al*., 1999). Serum COMP levels in patients with OA are substantially higher from those in normal donors (Clark AG *et.al*., 1999), and COMP levels can reflect disease severity and multiple joint involvement (Clark AG *et.al*., 1999). The role of type II collagen in eliciting T cell responses in patients with OA also needs to be further studied. T cells from the peripheral blood or synovial fluid of patients with RA recognize type II collagen and its immunodominant peptide.

8. Decreased expression of the CD3 ζ-chain in T cells infiltrating the synovial membrane of patients with OA: The CD3 ζ-chain is one of the CD3 proteins and is part of the T cell signal transduction cascade that is initiated by engagement of the TCR by appropriate antigenic epitopes and culminates in T cell activation and proliferation (Sakkas L and Chris D ,2007)

Involvement of B lymphocytes in Osteoarthritis

B cells along with other cell types have been reported to be present in the inflamed synovium in OA patients (Revell M et.al., 1988). A clonal analysis of B cells in OA synovium revealed their oligoclonal nature suggesting an antigen driven activation instead of non-antigenic activation

(Shiokawa S. *et.al*., 2001). Moreover, several studies found antibodies against cartilage components highlighting the activation of humoral adaptive immune response in OA (Mollenhauer J. *et.al*., 1988). Similarly, autoantibodies were found in OA patients against cartilage derived proteins osteopontin (Sakata M. *et.al*., 2001), cartilage intermediate layer protein (CILP) (Tsuruha J *et.al*., 2001), YKL-39, fibulin-4 (Xiang Y *et.al* 2004) and collagen. Anti-CCP antibodies were detected in 7 out of 136 OA patients (Du H. *et.al*., 2005), while another group also detected them in OA patients but at significantly lower levels compared to rheumatoid arthritis RA patients (Caspi D *et.al*.,2006). Antibodies against native G1 domain of aggrecan core protein were found in synovial fluid of OA patients (Rowley MJ et.al., 1996). Using proteomic approach, Xiang et al identified triosephosphate isomerase (TPI) as an important antigen with autoantibodies present specifically in OA but not in RA (Xiang Y *et.al* 2004). Other studies have reported autoantibodies in animal models of OA including horses (Niebauer GW *et.al*., 1988) and dogs (Cox E *et.al*., 2011). The role of the autoantibodies against cartilage components in development of OA has been further highlighted by studies showing their deposition and cytotoxic effects on cartilage, which may be one of the mechanisms playing important role in cartilage degeneration in OA (Abdul H. and Tariq M. 2013).

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