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Discussion on the involvement of T and B lymphocytes in the pathogenesis and progression of osteomyelitis and osteoarthritis

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OSTEOARTHRITIS

Introduction

Osteoarthritis (OA) is a chronic disease and 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 (Abdul and Tariq 2013). Many factors- some modifiable- contribute to an increased risk of OA and include obesity, genetics, aging and trauma to the joint. In most patients without a strong genetic predisposition, OA is thought to start 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 (Abdul and Tariq 2013). 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 (Abdul and Tariq 2013). Involvement of an immune response, both innate and adaptive, in OA is now widely accepted based on the following evidence: (Abdul and Tariq 2013).

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

Key role of complement activation in OA synovium has been identified (Abdul and Tariq 2013).

T lymphocytes in the pathogenesis and progression of osteoarthritis

Osteoarthritis (OA) is the most common type of arthritis (Lazaros *et al.,* 2007). The prevalence of symptomatic OA is at least12.1% in both sexes, whereas the prevalence of radio-graphically defined OA is much higher and increases with age (Lazaros *et al.,* 2007) OA is a heterogeneous disease, and its classification leaves much to be desired. Primary OA, which has no apparent predisposing factor, and secondary OA, in which the

patient has a prior trauma or condition related to OA, are the 2 most common subsets. Primary OA is called generalized OA when it affects many joints, nodal OA when it exhibits as nodes over interphalangeal joints and erosive inflammatory OA when it exhibits as erosions in distal interphalangeal joints (Lazaros *et al.,* 2007). Erosive inflammatory arthritis, which is characterized by flares of inflammation in joints and the presence of inflammation markers in peripheral blood, may represent the far end of the spectrum of generalized OA. Current treatments for OA are purely palliative, and the need for novel therapies is obvious. The etiology of primary OA is not known. Un-identified genetic factors have been implicated in the development of OA and a genetic component is supported by studies of families and twins (Lazaros *et al.,* 2007). Clonal chromosome aberrations, such as the gain of chromosomes were observed in the synovial membrane of certain patients with OA (Lazaros *et al.,* 2007). Alpha1-antitrypsin 1-antichymotrypsin, gene polymorphisms, and HLA alleles have been associated with generalized OA, whereas type II precollege gene polymorphisms have been associated with precocious OA with mild chondrodysplasia (Lazaros *et al.,* 2007)

T cells may play an important role in the pathogenesis and progression of OA as follows.

1**. CD3 T cells infiltrate the synovial membrane of patients with OA**

Several groups of investigators, including our own have reported the 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 per vascular nodular (Lazaros *et al.,* 2007). We have observed angiocentric infiltrates composed primarily of CD3 T cells in the synovial membrane of patients with OA, in a pattern similar to that observed in RA (ref. 25, and Sakkas LI, et al: unpublished results).Tran mural CD3T cells infiltrating the vessel wall were evident, although they were located mainly in perivascular areas. Many vessels were compressed and occluded, and endothelial cells were strongly positive forE-selectin, in a manner similar to that observed in RA (ref. 25, and Sakkas LI, et al: unpublished results). All of these observations in patients with OA arein addition to the findings in patients with the relatively uncommon type of erosive inflammatory disease, which clearly shows a strong inflammatory component (Lazaros *et al.,* 2007). In certain patients with OA, the MNC infiltrates resemble those observed in the synovial membrane of patients with RA (Lazaros *et al.,* 2007).
Nodular lymphocytic aggregates were observed in 14% of patients with early OA and in37% (22) to 65% of patients with advanced OA at the time of joint replacement surgery (Lazaros *et al.,* 2007). The presence of T cells may play an important role in the pathogenesis and progression of OA as follows.1. CD3 T cells infiltrate the synovial membrane of patients with OA. Several groups of investigators including our own have reported the presence of mononuclear cell (MNC) infiltrates consist-ing of T cells and macrophages in the synovial membrane of 50% of patients with OA. MNC infiltrates may be diffuse or perivascular nodular, We have observed angiocentric infiltrates composed primarily of CD3 T cells in the synovial membrane of patients with OA, in a

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2**. T cells infiltrating the synovial membrane ofpatients with OA express early, intermediate, and late activation antigens.**

We previously demonstrated that in the majority of patients with advanced OA, T cells infiltrating the synovial membrane express early activation antigens (CD69), intermediate activation antigens(CD25, CD38), and late activation antigens (CD45RO,HLA class II) (Lazaros *et al.,* 2007). 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 (Lazaros *et al.,* 2007). Although it could be argued that 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 (Lazaros *et al.,* 2007). CD38 and the CD43, which are detected in the synovial membrane of patients with OA mediate adhesion to vascular endothelium and binding to intercellular adhesion molecule 1 (ICAM-1), respectively. Leukocytes and endothelial adhesion molecules are also ex-pressed in the synovial membrane of patients with OA, although to a lesser degree than in patients with RA. B cells are also activated in patients with OA (Lazaros *et al.,* 2007)

3. HLA association of OA. Several studies havedemonstrated associations of OA with HLA class I andHLA class II alleles.

Studies on generalized OA revealedan association with HLA–B8 (Lazaros *et al.,* 2007). This association may not be primary, because HLA–B8 is in linkage disequilibrium with DR3. Another study in Japanese patients with generalized OA revealed an association with HLA–Cw4 (Lazaros *et al.,* 2007). The frequencies of the HLA–DRB10101, 0401, 0405, 1001, and 1402 alleles, which have been reported to be associated with RA, were not significantly different in patients with generalized OA compared with control subjects (Lazaros *et al.,* 2007). H and OA was found to be associated with the haplotype HLA–A1;B8 and haplotypes HLA–B35;DQ1,HLA–B40;DQ1, and HLA–DR2;DQ1 (Lazaros *et al.,* 2007). An association of dysplastic hip OA with HLA–DR4 was observed (Lazaros *et al.,* 2007). An association of the HLA–DRB102 allele was found to be a risk factor for the development of distal interphalangeal OA (Lazaros *et al.,* 2007). Similarly, in another study, an association of the HLA–DRB102 allele with OA was identified in a cohort of 106 patients, whereas the DR5allele was negatively associated with the disease

(Lazaros *et al.,* 2007). This HLA class II association of OA further supports the concept that OA, at least in certain patients, may be atrimolecular-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) (Lazaros *et al.,* 2007). Physical interaction between chondrocytes and T cells is conceivable, because cartilage fragments, which are mechanically shaved from the cartilage surface, are frequently found in the synovial membrane of patientswith OA (27). Proliferative responses in vitro of peri-pheral blood T cells from patients with OA to autologous chondrocytes were significantly higher compared with those of T cells from normal control subjects (Lazaros *et al.,* 2007). T cells derived from the peripheral blood or synovial fluid of patients with OA responded to membranepreparations of autologous chondrocytes and fibroblasts by proliferation (82). These T cell responses are monocyte dependent, suggesting an antigen-specific immune response (Lazaros *et al.,* 2007).

B lymphocytes in the pathogenesis and progression of osteoarthritis

Cellular infiltrates in the inflamed OA synovium have been reported to contain activated B cells along with other cell types (Abdul and Tariq 2013). A clonal analysis of B cells in OA synovium revealed their oligoclonal nature suggesting an antigen driven activation instead of non-antigenic activation (Abdul and Tariq 2013). Moreover, several studies found antibodies against cartilage components highlighting the activation of humoral adaptive immune response in OA. When cartilage cell surface proteins were used as substrate in an ELISA and sera from OA patients were applied, an elevated antibody titer was detected compared to controls (Abdul and Tariq 2013). Similarly, auto antibodies were found in OA patients against cartilage derived proteins osteopontin, cartilage intermediate layer protein (CILP), YKL-39, fibulin-4 and collagen. Anti-CCP antibodies were detected in 7 out of 136 OA patients, while another group also detected them in OA patients but at significantly lower levels compared to RA patients (Abdul and Tariq 2013). Antibodies against native G1 domain of aggrecan core protein were found in synovial fluid of OA patients (Abdul and Tariq 2013). Using proteomic approach, Xiang et al identified triosephosphate isomerase (TPI) as an important antigen with autoantibodies present specifically in OA but not in RA (Abdul and Tariq 2013). Other studies have reported autoantibodies in animal models of OA including horses and dogs (Abdul and Tariq 2013). The role of the auto antibodies 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 and Tariq 2013).

OSTEOMYELITIS

Introduction

The term Osteomyelitis encompasses a broad group of infectious diseases characterized by infection of the bone and/or bone marrow. The pathogenesis of these diseases can follow acute, sub acute or chronic courses and involves a range of contributory host and pathogen factors. A commonly used aetiological classification distinguishes between three types of Osteomyelitis: acute or chronic haematogenous disease seeded by organisms in the bloodstream, local spread from a contiguous source of infection and secondary Osteomyelitis related to vascular insufficiency. Acute haematogenous Osteomyelitis acute haematogenous Osteomyelitis refers to infection of bone resulting from bacteria in the bloodstream. This is seen most often in children, with initial infection thought to occur in the richly vascularised metaphyseal region (Gutierrez, 2005). Children are thought to experience frequent episodes of bacteraemia, often with no apparent symptoms, leading to seeding and development of osteomyelitis (Conrad, 2010). The pathogenesis of this process has been theoretically described. Inoculation of the metaphyseal vessels occurs at the transition point from the arteriolar vessels to the venous sinusoids, slowing blood flow and increasing vascular turbulence (Jansson et al., 2009). These sites of turbulence may be predisposed to bacterial infection by providing an opportunity for local invasion. Although rarely seen in developed countries, haematogenous osteomyelitis may take on a chronic course within bone if left untreated. Sequelae of this devastating condition may include chronic sinuses with exposed bone, loss of structural integrity and growth disturbances (Beckles et al., 2010). Local trauma to bone in the setting of bacteraemia may also be a contributing factor. Animal studies have shown significantly increased rates of haematogenous osteomyelitis when direct injury to bone was combined with intravenous bacterial seeding. (Kabak et al., 1999; Morrissy & Haynes, 1989).

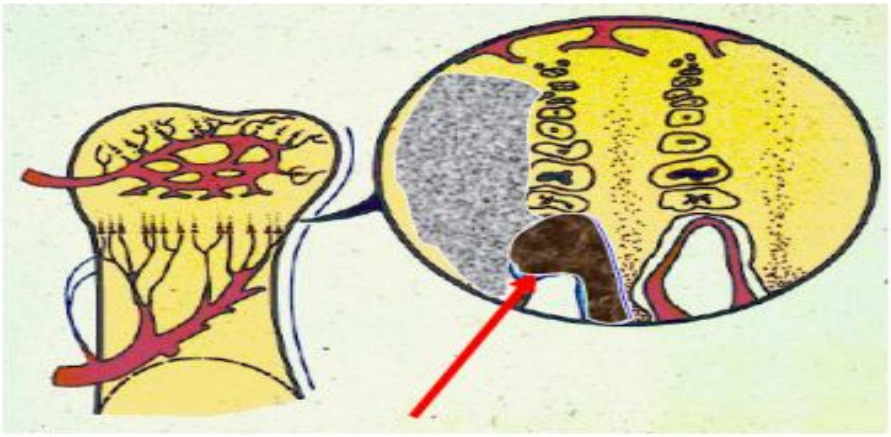

Fig. 1. Schematic drawing showing the vascular supply to the physis. The callout represents a detailed view of the physis. The red arrow indicates an area of transition. These transitional zones show increased turbulence and allow for local invasion. (Image used with permission from Dr. Kaye Wilkins)

Role of osteoblasts

The skeleton is a dynamic organ system, in a state of perpetual turnover which is continually remodelled by the actions of two cell types (Henderson & Nair, 2003). Osteoblasts are responsible for the deposition of bone matrix; they are found on bone surfaces and are derived from mesenchymal osteoprogenitor cells. These cells secrete osteoid, a mixture of bone matrix proteins primarily made up of type I collagen (over 90%), proteoglycans such as decorin and biglycan, glycoproteins such as fibronectin, osteonectin and tenascin-C, osteopontin, osteocalcin and bone sialoprotein, oriented along stress lines (Mackie, 2003).The opposing action of bone matrix removal is performed by osteoclasts, multinucleate cells that are derived from the macrophage-monocyte lineage. These cells express large quantities of a vacuolar-type H(+)-ATPase on their cell surface, along with chloride channel 7 (ClC 7) enabling localized hydrochloric acid secretion into a closed compartment, known as the resorption lacuna, and subsequent solubilization of bone mineral (Blair et al., 1989). The balance of activity between these two cell types is crucial to maintaining the proper homeostasis of bone turnover, and any shift in the relative levels of osteoblast and osteoclast activity can result in bone pathology (Henderson & Nair, 2003). Infection with a pathogen such as S. aureus is capable of stimulating such a shift, mediated in part by induction of an inflammatory response. There is an intimate interaction between the two cell types, with osteoblasts interpreting the majority of extracellular signals and subsequently modulating osteoclast differentiation and function (Henderson & Nair, 2003; Matsuo & Irie, 2008). Interaction between the RANK (receptor activator for nuclear factor κB) receptor, expressed by osteoclast precursors, and its cognate ligand, RANKL, expressed by osteoblasts is essential for osteoclastogenesis (Matsuo & Irie, 2008). Osteoprotegrin (OPG) is an endogenous inhibitor of RANKL signaling, functioning as a decoy receptor that binds to RANKL and prevents its association with RANK (Wada et al., 2006).

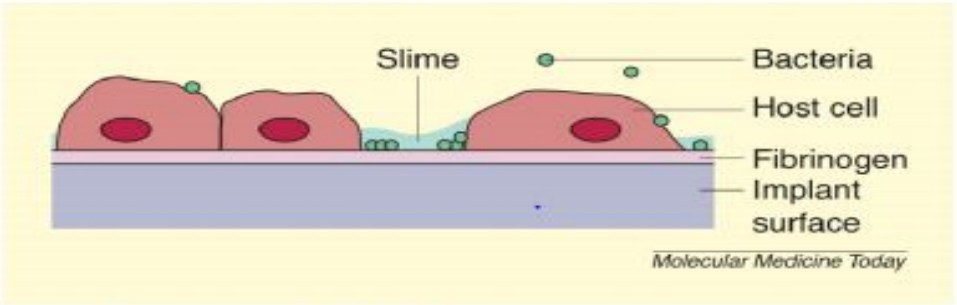

Fig. 3. Adherence of contaminating bacteria to implant surfaces competes with attachment of host cells. The implant surface soon becomes covered with plasma proteins, mainly fibrinogen, to which both host cells and bacteria can bind. In this 'race for the surface', bacteria are often the winners. Secondary to adherence to fibrinogen, staphylococci (mainly *S. epidermidis*) produce slime, further promoting adherence. Early intervention by blocking primary bacterial adherence would favour eukaryotic cells in the race. The slimy polysaccharide prevents phagocytosis and protects the bacteria from antibiotics. Reprinted from Flock, J.I., *Extracellular-matrix-binding proteins as targets for the prevention of Staphylococcus aureus infections*. Mol Med Today, 1999. 5(12): p. 532-7 with permission from Elsevier.

T lymphocytes in the pathogenesis and progression of Osteomyelitis

The goal of immune responses in infectious diseases is to eliminate pathogens through inflammatory reactions without collateral damage (Shreemanta *et al.,* 2015). T cells are not only the key mediators of adaptive immune responses, but they also orchestrate the delicate balance of immune responses between nonproductive and exaggerated inflammation. $CD4^+$ antigen-specific responses are found in humans 3–8 weeks following infection with *Mtb*, corroborated by the tuberculin skin test or interferon gamma (IFN-γ) release assay (IGRA) in humans. The role of $CD4^+$ cells, as well as interleukin (IL) 12 and IFN-γ, have been well documented by studies of the syndrome of Mendelian susceptibility to mycobacterial diseases, defined by a selective vulnerability to weakly virulent mycobacterial species (BCG and environmental mycobacteria) due to mutations in the IL-12 and IFN-γ receptors . Reactivation of latent infection with *Mtb* to clinical disease during TNF-α antagonist therapy in the first year of treatment suggests that TNF-α contributes to contain *Mtb* infection, which had been observed previously in murine models; TNF-α antagonist therapy also removes terminally differentiated TNF-α^+ ($CD45RA^+CCR7^-$) immune effector $CD8^+$ T cells, which underlines the role of *Mtb*-specific $CD8^+$ T cells in clinical tuberculosis, along with the observation that $CD8^+$ immune effector functions, including cytokine production and cytotoxic abilities, may be impaired. Concepts in targeted cellular therapy that are already used in clinical trials for viral targets or malignant cells may cross fertilize directed cellular therapy for the treatment of tuberculosis (Shreemanta *et al.,* 2015)

NATURE IMMUNE EFFECTOR T CELLS

The nature and specificity of the T-cell receptor (TCR), as well as the phenotype and function of the recipient effector cell population, appear to be crucial for clinically relevant responses. Immunopathogenesis of human tuberculosis is orchestrated by multiple players (Table 1) in dynamic cascades, and the outcome depends on these balances between several subsets of immune cells as well as a number of cytokines and chemokines. Too little inflammation or too much inflammation can lead to detrimental effects by allowing *Mtb* to multiply and thrive or exaggerated immune response to be pathogenic to the host, respectively, whereas the right balance determines the immune response to win the race. For instance, terminally differentiated T cells may be used for immediate immune effector functions, yet long-term memory responses (usually defined by the cell surface markers CD45RA, CCR7, and CD62L) are required to contain pathogens or transformed cells.

Early differentiating stem-cell memory T cells (T_{SCM}), precursors of other memory cells including central memory T cells (T_{CM}), have enhanced self-renewal capacity and multipotency. Human T_{SCM} express high levels of CD95, CXCR3, CD122, and LFA-1 and are distinct from central T_{CM} in terms of surface markers, tissue localization, cytokine production, and in vivo turnover. This antigen-specific subset is preferentially localized to lymph nodes and virtually absent from mucosal surface; it is generated in the acute phase of viral infection and persists beyond removal of the antigen contributing in supporting long-term cellular immunity in vivo(Shreemanta *et al.,* 2015). Therefore, the induction or adoptive transfer of these T-cell populations may be beneficial in anti-*Mtb*–directed immune responses. T_{SCM} have been demonstrated to persist, while preserving their precursor potential in bone marrow–transplanted patients for up to 12 years after infusion of gene-corrected hematopoietic stem cells, or mature lymphocytes that were tracked concerning their fate and activity (Shreemanta *et al.,* 2015). Antigen-specific T_{SCM} can differentiate directly from naive precursors, correlating with IL-7 serum levels. T_{SCM} may be achieved by pharmacological activation of the WNT (wingless type, signaling molecule) pathway (Shreemanta *et al.,* 2015). Alternate ways are being explored to achieve such a phenotype, for instance, using signaling inhibitors, for example, with inhibition of the AKT-1 signaling pathway (Shreemanta *et al.,* 2015). The nature of antigen-specific immune cells, the anatomical localization, and homing

patterns are crucial to mediate clinically relevant effects: T cells infused for adoptive therapies are trapped in the lungs where they encounter first a microcapillary network; inflammatory signals in case of tuberculosis would make intravenous application simple as T-cells are directly delivered to the lung, the first passage site. This also has an inherent risk of a “cytokine storm” once T cells encounter their nominal target antigen(s) (Shreemanta *et al.,* 2015)

B lymphocytes in the pathogenesis and progression of Osteomyelitis

Acute Osteomyelitis presents as a suppurative infection accompanied by edema (Jason and Shirtliff, 2009), vascular congestion, and small vessel thrombosis (Jason and Shirtliff, 2009). 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. 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 from 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.20 Bacteria have been shown to persist within glycocalyx-enclosed microcolonies adherent to the bone and to prosthetic devices in cases of osteomyelitis. (Jason and Shirtliff, 2009). 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 (Jason and Shirtliff, 2009). It has been reported that 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 (Jason and Shirtliff, 2009). 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 (Jason and Shirtliff, 2009).

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 (Jason and Shirtliff, 2009). 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. Endosteal new bone may proliferate and obstruct the medullary canal (Jason and Shirtliff, 2009). 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 (Jason and Shirtliff, 2009)

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