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ASSIGNMENT QUESTION: DISCUSS THE INVOVLEMENT OF T AND B LYMPHOCYTE IN THE PATHOGENESIS AND PROGRESSION OF OSTEOMYELITIS AND OSTEOARTHRITIS.

ANSWER.

Increasing numbers of patients currently benefit from joint prosthesis, due to ageing, trauma and improved surgical techniques . However, bone infection (BI) may compli- cate orthopaedic surgery, even when there are no prosthetic implants . Infection of total hip arthroplasty or total knee arthroplasty occurs with an incidence of 1·5–2·5% for primary interventions, but higher rates (2–20%) have been reported after revision procedures (Horwood NJ, Kartsogiannis V, 1999). BI, or osteomyelitis, is an inflammatory process accom- panied by bone destruction. While in industrial countries the infecting microorganisms are usually Staphylococcus species, species such as Mycobacterium tuberculosis and Sal- monella typhi are more common in other parts of the world . It is noteworthy that bone resorption is associated with infection, regardless of the causative microbiological agent(Potempa J, Banbula A, 2000). The role of immune activation at the site of infection is unclear, although it is thought to participate in bone resorption, as suggested by experimental and human studies, particularly in the case of periodontitis (Li H, Hong S, Qian J, Zheng Y, Yang J, Yi Q, 2010). Osteoimmunology is an interdisciplinary research field focused on the molecular understanding of the interplay between the immune and skeletal systems. T lymphocytes and their products have been recognized as key regulators of osteoclast (OC) and osteoblast (OB) formation, lifespan and activity . Interestingly, OC shows some characteris- tics of antigen-presenting cells (APC) in experimental models of inflammatory diseases (Wyzga N, Varghese S, et al, 2004), which imply an inter- action of OC with T cells. Accordingly, 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) . 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 [10]. Conversely, CD8+ T cells did not support OC differen- tiation, but rather inhibited OC differentiation/activation induced by RANKL . Of note, experimental models used CD3/CD28 stimula- tion to study lymphocyte–OC interactions [7, 9, 10]. However, co-stimulatory molecules, CD28, CD80, CD86, cytotoxic T-lymphocyte antigen-4 (CTLA-4)/CD152, CD40 and CD40L showed major alterations of expression in bac- terial infection, which may be responsible for modulating cellular interactions (Pessler F, Dai L, Diaz-Torne C et al,2008). Interestingly, analysis of synovium of patients with chronic septic arthritis showed dramatic T cell proliferation. This dramatic proliferation of T cells suggests the occurrence of some major cellular interactions . Among other key immunological features studied in osteoimmunology is apoptosis of CD4 T cells, for which researchers have suggested an inverse CD4/CD8 ratio observed in patients with aseptic loosening of prosthesis . Apoptosis might be related to regulatory T cells (Tregs), which are specialized to limit the magnitude of T cell effec- tor responses (Singh N, Yamamoto M, Takami M et al, 2005). To the best of our knowledge, all these T cell features have never been reported previously in human BI. Thus, our aim was to characterize the T cell phenotype infiltrating bacterial infected bones, focusing on the expression of co-stimulatory molecules. patients; the number of patients included in a particular set of data is specified in the text. Concerning blood experimentation, 12 healthy controls (20–25 years), four males and eight females, were from the hospital staff. Blood was collected in a tube containing acid citrate dextrose solution.

T CeLLS AND OSTEOARTHRITIS (OA) Analysis of enzyme-linked immunosorbent assay (ELISA) data has shown that compared with age-matched healthy controls, patients with OA show higher levels of the soluble form of CD4 (sCD4) in their serum. This suggests that peripheral T helper (Th) cells are involved in the pathogenesis of OA (Ponchel F, Burska AN et al, 2015). Similarly, when stimulated with phorbol myristate acetate (PMA) and ionomycin, peripheral mononuclear cells from OA patients showed a higher expression of CD4 and CD8 markers than their counterparts from healthy controls. Indeed, the ratio of CD4+/CD8+ in the blood of OA patients is higher than that in the blood of healthy controls, although healthy controls and OA patients have fairly similar numbers of CD4+ and CD8+ T cells in their blood (Hugle T, Geurts J, 2016). Further evidence of the involvement of peripheral T cells in the pathogenesis of OA was provided by the discovery that the response to autologous chondrocytes of peripheral T cells isolated from OA patients is greater than of peripheral T cells isolated from controls and that this response is partially blocked by antibodies against human leukocyte antigen (HLA) classes I and II, CD4, and CD8 . Interestingly, T cells in a subset of OA patients were found to recognize the peptides representing amino acid regions 16–39 and 263–282 of human cartilage proteoglycan aggrecan (PG), and peripheral blood mononuclear cells from these PG-reactive OA patients showed an increased production of pro-inflammatory cytokines/chemokines in response to PG peptide stimulation . Based on these compelling findings, the autoimmune responses of peripheral T cells may aid understand- ing of immune-mediated mechanisms in OA. 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 (Withrow J, Murphy C, Liu Y, Hunter M et al, 2016). When stimulated with PMA and ionomycin, mononuclear cells from the synovial fluid of OA patients showed a high expression of CD4 and CD8 markers. These compelling results suggested that T cells in the synovial fluid are associated with the pathogenesis of OA. This conclusion was supported by subsequent investigations. For example, the percentage of T cells in the synovial fluid of OA patients was found to be significantly higher than that in their peripheral blood, and T cells in the synovial fluid of OA patients expressed class II HLA (an indicator of activated T cells) (Fonseca JE, Edwards JC, Blades S, 2013). The percentages of CD4+ and CD8+ cells in the synovial fluid of OA patients were even similar to those found in RA patients. 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. For example, synovial tissue extracted from OA patients displayed perivascular CD3+ T cell infiltration at an early stage. Similarly, using immunohistochemical analysis, CD3+, CD4+, and CD8+ T cells were detected predominantly in the sublining 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 sublining layer was detected more strongly in OA patients than in normal subjects. 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 sublining layer of patients with OA than in that of normal subjects. Indeed, the medial synovium of patients with knee OA has been shown to contain more CD4+ T cells than the lateral synovium (Ishii H, Tanaka H, Katoh K, et al, 2002). Interestingly, synovial aggregates from OA patients express CD80, an induc- ible costimulatory ligand involved in T-cell activation, suggesting that synovial aggregates in OA patients are areas of antigen recognition and T-cell activation. Similarly,

researchers investigating 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. In addition, 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. 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 . Interestingly, OA patients older than 75 have higher percentages of CD3+, CD4+, and CD8+ cells in their synovial membranes than OA patients younger than 75. This may suggest that age is among the risk factors for OA. Collectively, significant abnormalities in the T-cell profile have been found in the peripheral blood, synovial fluid, and synovial membranes of OA patients. Based on these findings, T cells are assumed to be associated with the pathogenesis of OA.

Th1 and OA Under the stimulation of interleukin (IL)-12, naïve CD4+ T cells differentiate into Th1 cells, which produce IL-2, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, lymphotoxins, and granulocyte-macrophage colony-stimulating factor. Most current evidence indicates that Th1 cells do not alter sig- nificantly on entering the peripheral blood of OA patients. For example, flow cytometry analysis has shown that there is little difference in the percentage of circulating Th1 cells (CD4+IFN-γ+ T cells) between OA patients and healthy controls. Similarly, no variation in either the percentage or the absolute number of circulating Th1 cells (CD4+IFN-γ+ T cells) has been found between patients with OA and healthy controls. However, in a study with 25 OA patients and 13 healthy controls, the number of circulating Th1 cells (IFN-γ+CD4+CD8− T cells) and the level of serum IFN-γ were found to be significantly higher in patients with OA than in healthy controls . The difference in the markers (CD4+IFN-γ+ vs. IFN-γ+CD4+CD8−) used in the two studies to define Th1 cells may account for this discrepancy. Another explanation may lie in the variation between OA patients, such as differences between the stages of OA. The alteration of the Th1 cell profile in the peripheral blood of OA patients thus requires further investigation. In contrast with the findings for peripheral blood, the synovial fluid of OA patients shows an increase in Th1 cells. Although early experiments suggested that the concentrations of IL-2, IFN- γ, and TNF-β in the synovial fluid of OA patients are below the limit of detection by ELISA analysis , reverse transcription polymerase chain reaction (RT-PCR) analysis has since revealed that cells from the synovial fluid of OA patients express IL-2 and IFN-γ when stimulated with PHA and ionomycin . Indeed, intracellular IFN-γ has been detected at higher levels in both CD4+ and CD8+ cells from the synovial fluid than in the periph- eral blood of OA patients . In addition, high concentrations of IL-1β and TNF-α have been observed in the synovial fluid of patients with OA, whereas these markers are below the limit of detection in healthy subjects .

Th1 cells can also be found in the synovial membranes of OA patients. For example, IL-2, IFN-γ, and their receptors are usu- ally detected in the synovial membranes of OA patients . Similarly, INF-γ+ cells have been detected in the synovial mem- branes of patients with OA, predominantly in the sublining layer of the synovium, although to a lesser degree than in RA patients . In a mouse model of OA induced by anterior cruciate ligament transection (ACLT), the expression of IFN-γ increased during OA onset (30 days after ACLT) and then decreased at a later stage of OA (90 days after ACLT) . Most importantly, a well-designed study showed that Th1 cells are predominant in both OA and RA joints . Indeed, the number of

IFN-γ+ cells in the synovium of patients with OA is approximately five times greater than that of IL-4+ cells . In summary, although the profile of Th1 cells in the periph- eral blood requires further analysis, Th1 cells have been shown to accumulate in the synovial fluid and synovial membranes of OA patients, which suggests that Th1 cells play important roles in the pathogenesis of OA. In addition, Th1 cell responses in the synovial fluid and synovial membranes of OA patients may be a marker of OA disease activity (Diaz-Torne C, Schumacher HR et al, 2007). Th2 and OA When stimulated by IL-4, naïve CD4+ T cells differentiate into Th2 cells. Through the production of IL-4, IL-5, IL-10, and IL-13, Th2 cells affect the function of B cells, dendritic cells, eosinophils, etc. and play important roles in the host's defense against multicellular parasites and in the pathogenesis of aller- gies . Most recent studies have shown that Th2 cells undergo limited alteration in the peripheral blood, synovial fluid, and synovial membranes of OA patients. For example, in a study of 18 OA patients, the IL-10 transcript was found in nearly all of the patients using competitive PCR analysis, whereas IL-4 and IL-5 were not detected in the synovial membranes of any of the patients. Similarly, the concentrations of IL-4 and IL-10 in the synovial fluid were below the limit of detection by ELISA analysis. Using flow cytometry analysis, low concentrations of Th2 cytokines such as IL-4 and IL-10 were detected in both the synovial fluid and the peripheral blood of OA patients . Although cells from the synovial fluid of OA patients stimulated with PHA and ionomycin expressed IL-10 at 48 h poststimula- tion, no signal for IL-4 was detected by RT-PCR analysis . The observed expression of IL-10 in OA patients' synovial membranes or synovial fluid cells may come from other cells, such as regula- tory T cells (Treg cells). Together, although these compelling findings suggest that Th2 responses play only a limited role in the pathogenesis of OA, further strong evidence is needed to support this hypothesis. Th9 and OA Th9 cells, recently defined as subsets of Th cells, preferentially produce IL-9 . Th9 cells facilitate immune responses against melanoma and intestinal worms and are closely associated with the immunopathology of allergic and autoim- mune responses, such as systemic lupus erythematosus (SLE), experimental autoimmune encephalitis, and systemic sclerosis . Th9 cells are also involved in the pathogenesis of arthritis(Pessler F, Chen LX, Dai L et al, 2008). For example, a high level of IL-9 has been detected in the peripheral blood and synovial fluid of patients with RA and patients with psoriatic arthritis (PsA), and the level of IL-9 in the synovial fluid is higher than that in the peripheral blood for RA and PsA patients . Similarly, activated CD3+ T cells from the peripheral blood and synovial fluid of patients with PsA or RA produce high levels of IL-9 . These results suggest that Th9 cells play critical roles in the pathogenesis of RA and PsA. Indeed, Th9 responses have also been observed in OA. For example, a high level of IL-9 has been detected in the peripheral blood and synovial fluid of OA patients, and the activation of purified CD3+ cells from the peripheral blood and synovial fluid of patients with OA produces a high level of IL-9, although lower than that observed in RA or PsA patients . Even more importantly, in a study with 25 OA patients and 13 healthy controls, the number of circulating Th9 cells and serum IL-9 level were found to be significantly higher in OA patients than in healthy controls (Rollin R, Marco F, Jover JA et al, 2008). This study also found that the number of circulating Th9 cells was positively associ- ated with the level of C-reactive protein in OA patients and that both the number of Th9 cells and the level of serum IL-9 were positively correlated with OA index . In summary, these well-designed experiments lead to the con- clusion that Th9 cells significantly shape the pathogenesis of OA, as well as that of RA and PsA; however, the Th9 response in the synovial membranes of OA

patients needs further investigation. In addition, serum IL-9 or the number of circulating Th9 cells may be a marker of OA disease activity. Th17 and OA Th17 cells secrete IL-17A (also known as IL-17), IL-17F, IL-21, and IL-22. Transform growth factor (TGF)-β, IL-6, IL-1β, and IL-23 have been reported to promote the differentiation of Th17 cells (Leheita O, Abed Elrazek NY et al, 2006) . Th17 cells provide protection against bacterial infec- tion and are associated with the development of autoimmune diseases via the recruitment of cells in the granulocyte lineage, especially neutrophils . Early investigations indicated that neither the percentages of circulating pure Th17 cells (CD4+ IFN-γ−IL-22−IL-17+ T cells) and Th17 cells (CD4+IL-17+ T cells) nor the level of serum IL-17 differed significantly between OA patients and healthy controls . Similarly, no variation in the percentage or absolute number of circulating Th17 cells or the IL-17 plasma level was found between patients with OA and healthy controls . These findings indicated that little altera- tion occurs in the Th17 cell profile in the peripheral blood of OA patients. However, later observations suggested otherwise (Lindblad S, Hedfors E, 2008). In a rat model of OA induced by the injection of papain and l-cysteine into the right knee joint, the OA rats were found to have a higher serum IL-17 level than the control rats . In addition, in a study with 25 OA patients and 13 healthy controls, the number of circulating Th17 cells and the level of serum IL-17 were found to be significantly higher in patients with OA than in healthy controls (47). As in the case of Th1 cells, variation in the markers used to define Th17 cells (CD4+IL-17+ vs. IL-17+CD4+CD8−) and the patients selected for investigation (e.g., diagnosis standard, disease index, patients' background) may account for this dis- crepancy. These controversial findings regarding Th17 cell profile in the peripheral blood of OA patients suggest that the roles of circulating Th17 cells in the pathogenesis of OA need further investigation. Nevertheless, it is widely accepted that Th17 cells are present in the synovial fluid and synovial membranes of OA patients (Revell PA, Mayston V, Lalor P, Mapp P, 1998). For example, in addition to the strong expression of IL-17 mRNA in the synovial membranes of OA patients , a high level of IL-17 has been measured in the synovial fluid of OA patients, whereas both are below the limit of detection in healthy subjects . In addition, Th17 cells have been detected in the joints of OA patients, albeit in smaller numbers than in RA joints . Collectively, these interesting results demonstrate the accu- mulation of Th17 cells in the synovial fluid and synovial tissue of OA patients; however, the exact role of Th17 cell response in the biology of OA needs further investigation. Th22 and OA Originally, IL-22 was regarded as a product of Th17 cells; however, recent evidence has indicated that a distinct subset of human skin CD4+ T cells (Th22) produces IL-22 but not IL-17 or IFN-γ . Increasing evidence has been provided for the involvement of Th22 cells in the biology of RA. For example, the percentage of Th22 cells is higher in RA patients than in healthy controls, and the percentage of Th22 cells is positively correlated with IL-22 expression in RA patients . In addition, the percentage of Th22 cells is positively correlated with both C-reactive protein levels and joint disease activity scores in RA patients . These compelling discoveries indicate that Th22 response is associated with the pathogenesis of RA and that blocking IL-22 expression may be a reasonable therapeutic strategy for RA(Kummer JA, Tak PP, Brinkman BM et al, 2006). Th22 cells are also involved in the biology of ankylosing spondylitis. Similar to the results for RA, the percent- age and absolute number of circulating Th22 cells were found to be elevated in patients with ankylosing spondylitis compared with healthy controls . Similarly, ELISA analysis revealed that the level of IL-22 in the plasma was higher in patients with ankylosing spondylitis than in healthy controls . However, Th22 cells seem to play a limited

role in the pathogenesis of OA. For example, compared with healthy controls, OA patients show no change in the percentage of circulating Th22 cells (CD4+ IFN-γ−IL-17−IL-22+ T cells) and the level of IL-22 in the plasma . Similarly, another independent experiment revealed that neither the percentage nor the absolute number of circulating Th22 cells, nor the plasma level of IL-22, differ between patients with OA and healthy controls . Collectively, unlike RA and ankylosing spondylitis, OA involves only a limited alteration of Th22 response in the peripheral blood; however, we lack data on the Th22 profile in the synovial fluid and synovial tissue of OA patients. Treg Cells and OA Under the influence of TGF-β, naïve T cells differentiate into Treg cells, which produce IL-10 and TGF-β (43, 72–74). Treg cells are important immunoregulators in many inflammatory and autoimmune diseases, as they modulate the secretion of anti-inflammatory cytokines and the expression of receptors for cytokines (Pettit AR, Ahern MJ, Zehntner S, 2004). For example, RA patients have a lower percentage of Treg cells at sites of synovial inflammation and in the peripheral blood , which may induce the downregula- tion of T-cell tolerance and exacerbate the inflammatory pro- cess. Increasing evidence has been provided that the profile of Treg cells in the peripheral blood, synovial fluid, and synovial membranes of OA patients is similar to that of RA patients. For example, the percentage and absolute number of Treg cells (CD4+ CD25+/highCD127−/low) in the peripheral blood, synovial fluid, and synovial membranes are similar in RA patients and OA patients, and Treg cells in both cases show greater accumulation in the syn- ovial fluid and synovial membranes than in the peripheral blood . In addition, Treg cells in the peripheral blood, synovial fluid, and synovial membranes of both OA patients and RA patients display a memory phenotype (CD45RO+RA−) . Neither does the activation status (CD69 and CD62L) nor the expression of markers associated with Treg function (CD152, CD154, CD274, CD279, and GITR) in the peripheral blood, synovial fluid, or synovial membranes differ between OA patients and RA patients . Those compelling results indicate that as in the case of RA, a decrease in Treg-cell responses is involved in the pathogenesis of OA. Indeed, (Ponchel et al, 2009) analyzed blood from 121 healthy controls and 114 OA patients and found that the OA patients had fewer Treg cells than the healthy controls after adjusting for age . Although the frequency of CD4+CD25+Foxp3+ Treg cells has been found to be elevated in the blood of OA patients, OA patients show lower IL-10 secretion from Treg cells and fewer Tim-3+ Treg cells in the blood . Similarly, in a rat model of OA induced by the injection of papain and l-cysteine into the right knee joint, the percentage of CD4+CD25+Foxp3+ Treg cells in the peripheral blood was significantly lower in the OA rats than in the control rats . In summary, a decrease in Treg-cell response may be involved in the pathogenesis of OA; however, the alteration of Treg-cell responses in the peripheral blood, synovial fluid, and synovial membranes of OA patients requires more comparative investiga- tion with age-matched healthy controls (Da RR, Qin Y, Baeten D, Zhang Y, 2007). Follicular Helper T (Tfh) Cells and OA Follicular helper T cells, located in the follicles of lymphoid tissue, induce B cells to produce immunoglobulins . Tfh cells express various distinguishing genes, such as CXCR5, PD-1, ICOS, CD40L, Bcl-6, and IL-21 . Increasing evidence has been provided for the influence of Tfh cells on the severity of autoimmune diseases, such as SLE and RA. For example, the number of circulating Tfh cells (CXCR5+ICOS+CD4+ cells or CXCR5+PD-1+CD4+ cells) has been shown to increase in a subset of SLE patients in line with the diversity and concentration of autoantibodies and SLE severity . Similarly, immunohistochemistry analysis has revealed spe- cific staining for CD4, CXCR5, and ICOS on infiltrating immune cells in the synovial tissues of RA patients, and

the presence of Tfh cells (CD4+CXCR5+ICOS+ T cells) in the synovial tissues of RA patients has been verified using both triple-fluorescence immu- nostaining and confocal laser scanning . This study provided evidence of the presence of Tfh cells in both SLE and RA patients, indicating the potentially important roles played by Tfh cells in the pathogenesis and progression of both diseases (Nakano S, Mishiro T, Takahara S et al, 2002). However, the results of immunohistochemistry analysis, triple-fluorescence

immunostaining, and confocal laser scanning revealed that Tfh cells are absent from the synovial tissues of OA patients . Yet, a recent investigation demonstrated the importance of Tfh cells to the pathogenesis and progression of OA (Lebre MC, Jongbloed SL, Tas SW, Smeets TJ et al, 2008). In the latter study, the frequency of ICOS+, PD-1+, and IL-21+ CXCR5+CD4+ T cells in the peripheral blood of 40 patients with OA and 13 healthy controls was examined by flow cytometry, and the concentra- tion of serum IL–21 was also determined. Compared with the healthy controls, the OA patients showed higher percentages of CXCR5+CD4+, PD-1+CXCR5+CD4+, ICOS+CXCR5+CD4+, and IL-21+CXCR5+CD4+ T cells . (Shan et al, 2001) also found that OA patients exhibited higher levels of serum IL-21 than healthy controls and, even more importantly, that the expression of IL-21+Tfh cells in OA patients was positively correlated with the disease activity of OA . The latter study suggests that Tfh cells play a critical role in the pathogenesis and progression of OA. However, further well-designed research is needed to characterize Tfh cell profile in the peripheral blood, synovial fluid, and synovial membranes of OA patients. Cytotoxic T Cells and OA The peripheral blood of OA patients has been analyzed using flow cytometry, revealing that patients with OA have significantly fewer CD8+ T cells and a higher CD4+:CD8+ ratio than healthy subjects . However, patients with OA have normal propor- tions of CD8+CD45RA+, CD8+CD29+, and CD8+S6F1+ cells in both their peripheral blood and their synovial fluid . These results indicate the alteration of peripheral CD8+ T cells in OA patients. Although CD8+ T cells can be found in the synovial membranes of OA patients, the major component of the T-cell infiltrate cannot. Most of the T cells found in the synovial mem- branes of patients with OA are helper T cells, whereas cytotoxic T cells occur sparsely in patients with OA (39, 86). Similarly, fewer CD8+ T cells than CD4+ T cells have been found in the lining, the sublining, and even the deep layer of the synovium of patients with OA (15). In addition, although both CD4+ and CD8+ T cells have been found in the synovial aggregates of OA patients, the aggregates contain a larger proportion of CD4+ T cells than of CD8+ T cells, and the CD8+ T cells are often located toward the periphery of the aggregates (Symons JA, McCulloch JF et al, 1991). CD8+ T cells play an important role in the pathogenesis of OA, although they are not the predominant T-cell type found in the synovial aggregates of OA patients. In mice with ACLT-induced OA, CD8+ T cells were activated once OA had been initiated, and the percentage of activated CD8+ T cells was significantly higher in the ACLT group than in the sham group during OA progression . In addition, the number of CD8+ T cells expressing tissue inhibi- tor of metalloproteinase-1 (TIMP-1) was found to be correlated with OA severity and inhibiting the expression of TIMP-1 in the joints retarded the progression of OA . Cartilage degenera- tion occurred more slowly in CD8+ T cell knockout mice than in wild-type mice . In summary, a significant alteration to CD8+ T cells has been observed in the peripheral blood, the synovial fluid, and the synovial membranes, and CD8+ T cells have been found to significantly shape the pathogenesis of OA, although they do not play the most important role in the process. T Memory (Tm) Cells and OA Once activated, most T cells undergo apoptosis; however, a minority persist as Tm cells.

An increasing number of researchers have begun to investigate the profile of Tm cells in the patho- genesis of OA. For example, although healthy individuals showed no difference in the percentages of CD45RO+CD4+ T cells and CD45RA+CD4+ T cells in the peripheral blood, more CD45RO+ cells than CD45RA+ cells were found in the peripheral blood of patients with OA . In patients with OA, the majority of CD4+ T cells in the synovial fluid and synovial tissue are CD45RO+ and CD45RA−, suggesting that an accumulation of CD45RO+ memory CD4+ T cells is a generalized phenomenon in OA joints (Hussein MR, Fathi NA, El-Din AM et al, 2008). Similarly, a study with 25 OA patients and 13 healthy controls revealed that the number of circulating CD4+CD45RO+ T cells was significantly higher in patients with OA than in healthy con- trols . Other evidence for the possible involvement of Tm cells in the pathogenesis of OA includes the detection of the regulated on activation, normal T cell expressed, and secreted chemokine (a potent chemoattractant for leukocytes, such as CD45RO+ memory T cells) and CD29 (a 1 integrin expressed by Tm cells) in the synovial fluid of OA patients (Sakata M, Masuko-Hongo K et al, 2003). In summary, CD45RO+ memory CD4+ T cells seem to be criti- cal to the biology of OA, yet their exact roles in the pathogenesis of OA have yet to be determined. Unconventional T Cells and OA Recent investigations have also highlighted the involvement of unconventional T cells in the pathogenesis of OA. For example, more and more evidence has been provided that γδ T cells are involved in the pathogenesis of RA. For example, the number of γδ T cells has been found to increase in the synovial membranes of RA patients , and γδ T cells in the synovial membranes have more and/or more avid Fc receptors for immunoglobulin G IgG in patients with RA compared with controls . Further research has shown that the majority of synovial γδ T cells in RA patients do not express Vγ9, Vδ2, or Vδ1-Jγδ1 . However, most recent studies have indicated that the number of γδ T cells in the synovial membranes of patients with OA does not increase . Immunohistochemical staining of synovial tissue with early-stage OA shows T-cell infiltration in the perivascular area, with the clonality of restricted T cell receptor usage in the V beta chain, which also indicates the minimal alteration of γδ T cells in OA patients. Recent studies have shown that the synovial membranes of OA patients express CD1 , which presents non-protein antigens to NKT cells, suggesting that CD1-restricted T cells may play a role in the pathogenesis of OA. Overall, although numerous studies of the involvement of conventional T cells in OA have been conducted, it will be useful to determine the importance to OA of unconventional T cells such as CD1-restricted T cells, MR1-restricted mucosal- associated invariant T cells, major histocompatibility complex class Ib-reactive T cells, and γδ T cells .

The pathogenesis of OA is still poorly under- stood, but multiple factors, including genetic, constitutional, and environmental factors are considered to play a part. One of the dominant clinical symptoms in decompensated OA is characterised by a non-infectious chronic inflammatory condition. Cellular and humoral immunity to collagen, cartilage proteoglycan, and chondrocyte surface antigens have been found in patients with OA.2–5 A histological examination of 38 OA synovial membranes showed that over half the OA synovial mem- branes contained a lymphoid infiltrate, that 17 showed lymphoid aggregates, and that in seven there were well formed lymphoid follicles (de Jong H, Berlo SE, Hombrink P et al, 2010). In most cases,HLA-DR+ cells were present in the subintimal region of the synovial membrane.1 These findings suggest that a local or systemic immune response, or both, may have a role in

certain aspects of the pathophysiology of OA. In all patients analysed, our µ and ã RT-PCR-SSCP analysis data indicate a clonal expansion of B cells in the OA synovial membrane. In addition, the µ RT-PCR-SSCP data, together with the sequence information, indicate the presence of common B cell clones at diVerent separated sites in the synovial membranes in four of six patients. Common B cell clones were also detected in the synovial membranes from both knee joints in one patient. Clonal expansion is unlikely to be driven by polyclonal activators such as super- antigens, which stimulate the B cell expressing

Table 1 Amino acid sequences of the CDR3 regions

Patient Sample VH N-DH-N JH

1 SM2 AR EISSYSSSYD YFDFW 2 SM1, 2 AG GGLQFLEWE DYW 3 SM1, 2 AR NFRSGDKGYN YW 4 SM1–3 AR GTSPGSYLIDF W 5 SM1 AR YSEPGTGRRAH FDYW 6 SM1, 3 AR LYGSSPKSAR YMDVW

members of the specific VH family, thus leading to the polyclonal expansion of these cells without conserving any particular CDR3 sequence. Rather, clonal expansion is a neces- sary feature of antigen driven immune re- sponses. Specific antigens expressed during Borrelia burgdorferi infection induce oligoclonal synovial Ig production.11 The clonal expansion of B cells has also been described in the salivary gland of Sjögren's syndrome.12 In the ã RT-PCR-SSCP analysis, BM and PBMCs showed a few faint and/or dominant bands except for the BM from patient 6. The PBMCs from patient 5 showed two bands in the µ RT-PCR-SSCP analysis. Two bands in PBMCs from patient 5 in the µ RT-PCR-SSCP analysis and two faint bands in BM from patient 1 in the ã RT-PCR-SSCP analysis were also present in the analyses of the synovial membranes. At present, we do not know whether the expanded B cell clones in the OA synovial membranes are generated in the synovium or whether they are generated in a secondary lymphoid organ and thereafter migrate to the synovial tissues. In both cases, it is possible that memory B cell clones and plasma cells which have the same antigen spe- cificity as the expanded B cell clones in the OA synovium may circulate in the PB and reside in the BM. When immune responses to joint components mainly occur in a secondary lym- phoid organ, clonal B cells may only be detected in BM and/or PB, but not in synovial membranes. The reason why oligoclonal bands in BM were found only in the ã RT-PCR-SSCP analysis is not clear. IgG producing clones may have a stronger tendency to reside in BM than IgM producing ones. The µ and ã RT-PCR- SSCP analyses of PBMCs and BM from healthy subjects showed only a smear pat- tern.89Oligoclonal bands in BM and PB from the patients with OA may be further evidence of the participation of immune responses in the pathogenetic processes of OA. Many autoantigens are targeted by the immune system in patients with RA. Most of these autoantigens are also targeted in patients with other autoimmune diseases. Oligoclonal B cell expansion seen in OA synovial membranes suggests that diVerent B cell reactivities are also present in OA joints and some of them, not just one reactive to a single autoantigen, may induce, sustain, and modify the disease course of OA. Locally synthesised Igs may contribute to the chronic inflammatory processes through the activation of complement and resident mononuclear phagocytes. Recent studies dis- closed that auto-reactive B cells play a part in the activation and diversification of auto-reactive T cells, probably as antigen presenting cells. It is tempting to consider that oligo-clonally expanded B cells in the synovial membranes activate T cells and thus sustain the chronic inflammation seen in OA joints.

Some clonal B cells are present only at one site in the synovial membrane. To generate such B cell clones, individual infiltrating B cells are considered to be independently stimulated by antigen presenting cells at diVerent locations to proliferate and produce separate clones. The common B cell clones at diVerent and separate sites in the synovial membrane may be generated in a secondary lymphoid organ and thus subsequently migrate to the synovial membrane, where they become activated and induced to proliferate. Further studies on the immune response and subsequent chronic inflammation in the OA synovial membrane will help us to obtain a better understanding of the pathogenesis of OA and thereby lead to the development of new treatment strategies for OA.

REFRENCE.

1. Nasser S. The incidence of sepsis after total hip replacement arthroplasty. Semin Arthroplasty 1994; 5:153–59.

2. Lew DP, Waldvogel FA. Osteomyelitis. N Engl J Med 1997; 363:999–1007.

3. Lew DP, Waldvogel FA. Osteomyelitis. Lancet 2004; 364:369–79.

4. Shirtliff ME, Cripps MW, Mader JT. Retrospective review of 728 patients with long bone osteomyelitis. American Society for Microbiology 99th General Meeting, Chicago. 1999. c410.

5. Potempa J, Banbula A, Travis J. Role of bacterial proteinases in matrix destruction and modulation of host responses. Periodontology 2000; 24:153–92.

6. Graves DT, Cochran D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. J Periodontol 2003; 74:391–401.

7. Horwood NJ, Kartsogiannis V, Quinn JM, Romas E, Martin TJ, Gillespie MT. Activated T lymphocytes support osteoclast forma- tion in vitro. Biochem Biophys Res Commun 1999; 265:144–50.

8. Li H, Hong S, Qian J, Zheng Y, Yang J, Yi Q. Cross talk between the bone and immune systems: osteoclasts function as antigen- presenting cells and activate CD4+ and CD8+ T cells. Blood 2010; 116:210–17.

9. Wyzga N, Varghese S, Wikel S, Canalis E, Sylvester FA. Effects of activated T cells on osteoclastogenesis depend on how they are activated. Bone 2004; 35:614–20.

10. Kong YY, Feige U, Sarosi I et al. Activated T-cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999; 402:304–09.

11. Buchwald ZS, Kiesel JR, DiPaolo R, Pagadala MS, Aurora R. Osteo- clast activated FoxP3+ CD8+ T-cells suppress bone resorption in vitro. PLOS ONE 2012; 7:e38199.

12. Cacere CR, Mendes-Giannini MJS, Fontes CJ, Kono A, Duarte AJS, Benard G. Altered expression of the costimulatory molecules CD80, CD86, CD152, PD-1 and ICOS on T-cells from paracoccidioidomycosis patients: lack of correlation with T-cell hyporesponsiveness. Clin Immunol 2008; 129:341–49.

13. Pessler F, Dai L, Diaz-Torne C et al. Increased angiogenesis and cellular proliferation as hallmarks of the synovium in chronic septic arthritis. Arthritis Rheum 2008; 59:1137–46.

14. Landgraeber S, von Knoch M, Löer F et al. Association between apoptosis and CD4+/CD8+ T-lymphocyte ratio in aseptic loosen- ing after total hip replacement. Int J Biol Sci 2009; 5:182–91.

15. Singh N, Yamamoto M, Takami M et al. CD4+CD25+ regulatory T cells resist a novel form of CD28− and fas-dependent p53-induced T cell apoptosis. J Immunol 2010; 184:94–104.

16. Ponchel F, Burska AN, Hensor EM, Raja R, Campbell M, Emery P, et al. Changes in peripheral blood immune cell composition in osteoarthritis. Osteoarthritis Cartilage (2015) 23(11):1870–8. doi:10.1016/j.joca.2015. 06.018

17. Hugle T, Geurts J. What drives osteoarthritis? – synovial versus subchondral bone pathology. Rheumatology (Oxford) (2016). doi:10.1093/rheumatology/ kew389

18. Withrow J, Murphy C, Liu Y, Hunter M, Fulzele S, Hamrick MW. Extracellular vesicles in the pathogenesis of rheumatoid arthritis and osteoarthritis. Arthritis Res Ther (2016) 18(1):286. doi:10.1186/s13075-016-1178-8

19. Fonseca JE, Edwards JC, Blades S, Goulding NJ. Macrophage subpopulations in rheumatoid synovium: reduced CD163 expression in CD4+ T lym- phocyte-rich microenvironments. Arthritis Rheum (2002) 46(5):1210–6. doi:10.1002/art.10207

20. Ishii H, Tanaka H, Katoh K, Nakamura H, Nagashima M, Yoshino S. Characterization of infiltrating T cells and Th1/Th2-type cytokines in the synovium of patients with osteoarthritis. Osteoarthritis Cartilage (2002) 10(4):277–81. doi:10.1053/joca.2001.0509

21. Diaz-Torne C, Schumacher HR, Yu X, Gomez-Vaquero C, Dai L, Chen LX, et al. Absence of histologic evidence of synovitis in patients with Gulf War veterans' illness with joint pain. Arthritis Rheum (2007) 57(7):1316–23. doi:10.1002/art.23006

22. Pessler F, Chen LX, Dai L, Gomez-Vaquero C, Diaz-Torne C, Paessler ME, et al. A histomorphometric analysis of synovial biopsies from individuals with Gulf War Veterans' Illness and joint pain compared to normal and osteoarthritis synovium. Clin Rheumatol (2008) 27(9):1127–34. doi:10.1007/ s10067-008-0878-0

23. Rollin R, Marco F, Jover JA, Garcia-Asenjo JA, Rodriguez L, Lopez-Duran L, et al. Early lymphocyte activation in the synovial microenvironment in patients with osteoarthritis: comparison with rheumatoid arthritis patients and healthy controls. Rheumatol Int (2008) 28(8):757–64. doi:10.1007/ s00296-008-0518-7

24. Leheita O, Abed Elrazek NY, Younes S, Mahmoud AZ. Lymphocytes sub- sets in osteoarthritis versus rheumatoid arthritis. Egypt J Immunol (2005) 12(2):113–24.

25. Lindblad S, Hedfors E. Arthroscopic and immunohistologic characterization of knee joint synovitis in osteoarthritis. Arthritis Rheum (1987) 30(10):1081– 8. doi:10.1002/art.1780301001

26. Revell PA, Mayston V, Lalor P, Mapp P. The synovial membrane in osteo- arthritis: a histological study including the characterisation of the cellular infiltrate present in inflammatory osteoarthritis using monoclonal antibod- ies. Ann Rheum Dis (1988) 47(4):300–7. doi:10.1136/ard.47.4.300

27. Kummer JA, Tak PP, Brinkman BM, van Tilborg AA, Kamp AM, Verweij CL, et al. Expression of granzymes A and B in synovial tissue from patients with rheumatoid arthritis and osteoarthritis. Clin Immunol Immunopathol (1994) 73(1):88–95. doi:10.1006/clin.1994.1173

28. Pettit AR, Ahern MJ, Zehntner S, Smith MD, Thomas R. Comparison of differentiated dendritic cell infiltration of autoimmune and osteoarthritis synovial tissue. Arthritis Rheum (2001) 44(1):105–10. doi:10.1002/ 1529-0131(200101)44:1<105:AID-ANR14>3.0.CO;2-3

29. Benito MJ, Veale DJ, FitzGerald O, van den Berg WB, Bresnihan B. Synovial tissue inflammation in early and late osteoarthritis. Ann Rheum Dis (2005) 64(9):1263–7. doi:10.1136/ard.2004.025270

30. Da RR, Qin Y, Baeten D, Zhang Y. B cell clonal expansion and somatic hypermutation of Ig variable heavy chain genes in the synovial membrane of patients with osteoarthritis. J Immunol (2007) 178(1):557–65. doi:10.4049/ jimmunol.178.1.557

31. Nakano S, Mishiro T, Takahara S, Yokoi H, Hamada D, Yukata K, et al. Distinct expression of mast cell tryptase and protease activated receptor-2 in synovia of rheumatoid arthritis and osteoarthritis. Clin Rheumatol (2007) 26(8):1284–92. doi:10.1007/s10067-006-0495-8

32. Lebre MC, Jongbloed SL, Tas SW, Smeets TJ, McInnes IB, Tak PP. Rheumatoid arthritis synovium contains two subsets of CD83-DC-LAMP- dendritic cells with distinct cytokine profiles. Am J Pathol (2008) 172(4):940–50. doi:10.2353/ajpath.2008.070703

33. Sakkas LI, Platsoucas CD. Role of T cells in the pathogenesis of osteoarthritis. Arthritis Rheum (2002) 46(11):3112–3. doi:10.1002/art.10483

34. Symons JA, McCulloch JF, Wood NC, Duff GW. Soluble CD4 in patients with rheumatoid arthritis and osteoarthritis. Clin Immunol Immunopathol (1991) 60(1):72–82. doi:10.1016/0090-1229(91)90113-O

35. Dolganiuc A, Stavaru C, Anghel M, Georgescu E, Chichos B, Olinescu A. Shift toward T lymphocytes with Th1 and Tc1 cytokine-secterion profile in the joints of patients with osteoarthritis. Roum Arch Microbiol Immunol (1999) 58(3–4):249–58.

36. Hussein MR, Fathi NA, El-Din AM, Hassan HI, Abdullah F, Al-Hakeem E, et al. Alterations of the CD4(+), CD8 (+) T cell subsets, interleukins-1beta, IL-10, IL-17, tumor necrosis factor-alpha and soluble intercellular adhe- sion molecule-1 in rheumatoid arthritis and osteoarthritis: preliminary observations. Pathol Oncol Res (2008) 14(3):321–8. doi:10.1007/s12253-008- 9016-1

37. Sakata M, Masuko-Hongo K, Nakamura H, Onuma H, Tsuruha JI, Aoki H, et al. Osteoarthritic articular chondrocytes stimulate autologous T cell responses in vitro. Clin Exp Rheumatol (2003) 21(6):704–10.

38. de Jong H, Berlo SE, Hombrink P, Otten HG, van Eden W, Lafeber FP, et al. Cartilage proteoglycan aggrecan epitopes induce proinflammatory autoreac- tive T-cell responses in rheumatoid arthritis and osteoarthritis. Ann Rheum Dis (2010) 69(1):255–62. doi:10.1136/ard.2008.103978

39. van de Putte LB, Meijer CJ, Lafeber GJ, Kleinjan R, Cats A. Lymphocytes in rheumatoid and nonrheumatoid synovial fluids. Nonspecificity of high T-cell and low B-cell percentages. Ann Rheum Dis (1975) 35(5):451–5. doi:10.1136/ ard.35.5.451

40. Haynes MK, Hume EL, Smith JB. Phenotypic characterization of inflamma- tory cells from osteoarthritic synovium and synovial fluids. Clin Immunol (2002) 105(3):315–25. doi:10.1006/clim.2002.5283

41. Nakamura H, Yoshino S, Kato T, Tsuruha J, Nishioka K. T-cell mediated inflammatory pathway in osteoarthritis. Osteoarthritis Cartilage (1999) 7(4):401–2. doi:10.1053/joca.1998.0224

42. Sanders ME, Makgoba MW, Sharrow SO, Stephany D, Springer TA, Young HA, et al. Human memory T lymphocytes express increased levels of three cell adhesion molecules (LFA-3, CD2, and LFA-1) and three other molecules (UCHL1, CDw29, and Pgp-1) and have enhanced IFN-gamma production. J Immunol (1988) 140(5):1401–7.

43. Sakkas LI, Scanzello C, Johanson N, Burkholder J, Mitra A, Salgame P, et al. T cells and T-cell cytokine transcripts in the synovial membrane in patients with osteoarthritis. Clin Diagn Lab Immunol (1998) 5(4):430–7.

44. Johnell O, Hulth A, Henricson A. T-lymphocyte subsets and HLA-DR- expressing cells in the osteoarthritic synovialis. Scand J Rheumatol (1985) 14(3):259–64. doi:10.3109/03009748509100403