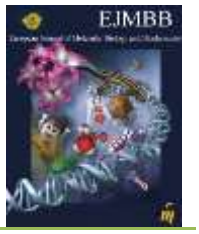
e - ISSN - 2348-2206
Print ISSN - 2348-2192

European Journal of Molecular Biology and Biochemistry

Journal homepage: www.mcmed.us/journal/ejmbb



MECHANISMS OF BRUCELLA ABORTUS-INDUCED OSTEOCLASTOGENESIS: IMPLICATIONS FOR BONE LOSS IN INFECTION

Sudha Parimala S*

Assistant Professor, Department of Pathology, Sree Balaji Medical College & Hospital, Chennai – 600044, Tamil Nadu, India.

Article Info

Received 03/11/2017
Revised 16/11/2017
Accepted 04/12/2017

Key words:- Brucella abortus, Osteoclastogenesis, T lymphocytes, Bone loss, Cytokines

ABSTRACT

The underlying mechanisms by which Brucella species induce bone loss remain unclear, posing challenges for effective infection control. This study investigates Brucella abortus-induced T lymphocyte (LT) responses and their role in osteoclastogenesis, a key process linked to bone density reduction. Brucella-induced inflammation activates LTs, promoting osteoclastogenesis in both in vitro and in vivo models. Culture supernatant from B. abortus-infected macrophages stimulates osteoclastogenesis in bone marrow-derived monocytes. CD4+ T cells are identified as mediators of osteoclastogenesis, and critical cytokines, RANKL and IL-17, secreted by B. abortus-activated T cells, play essential roles in this process. The study further reveals the involvement of IL-6 in promoting LT-derived pro-osteoclastogenic IL-17 during Brucella infection. Additionally, IL-17 indirectly induces osteoclastogenesis through the induction of tumor necrosis factor-α. These findings provide valuable insights into the complex interplay between Brucella infection, T cell activation, and bone loss, emphasizing the significance of understanding these mechanisms for developing targeted therapeutic strategies.

INTRODUCTION

Several forms of osteoarticular brucellosis are associated with bone loss, including arthritis in the knee, spondylitis, and peripheral joints [1–5]. In Brucella species, bone loss is caused by unknown mechanisms. In response to *Brucella abortus* infection, however, we have reported a potentially mechanism of immune-mediated inflammatory bone loss [6-11]. We found that macrophages play a crucial role in responding to *B. ovatus* and osteoclastogenesis. A chronic inflammatory bone disease also causes bone loss due to cell recruitment. Tumor necrosis factor also plays a role in bone loss, IL-1, and IL-6, as well as pro-inflammatory cytokines, has been implicated in disease progression and bone loss.

A number of studies have linked activated T and B lymphocytes to bone resorption [12–14]. In particular, activated T cells undermine bone homeostasis when estrogen levels are low or when chronic inflammation is present.

Corresponding Author
Sudha Parimala S

Other T-cell-derived cytokines, such as interferon and IL-4, IL-17, IL-10, and IL-24, inhibit osteoclastogenesis as well [15]. A study found that IL-17-producing T-helper (Th17) cells promote osteoclastogenesis. Several inflammatory cytokines, including IL-17, also promote osteoclastogenic activity. Inflammatory bone loss is mediated by osteoclasts and T cells interact.

There are typically bone destruction of varying degrees lymphocyte infiltration associated with lesions of the bones and joints caused by chronic brucellar inflammation. Furthermore, lymphocytes are commonly found in synovial tissue. The release of pro-inflammatory cytokines by Brucella species was demonstrated in a recent study of human osteoblasts infected and survived by Brucella species and chemokines implicated in osteoarticular manifestations. As a result, osteoblasts and monocytes were able to interact more effectively after B. Adenocarcinoma abortus infection. [6, 16] As a result, it is plausible that infiltrating T cells may be activated and stimulated to produce osteoclasts. Bone is degraded only

by these cells, in combination with the bacterium generates an inflammatory microenvironment. As a result, Model developed in vitro to test whether Brucella-infected macrophage culture supernatants (CSs) could induce osteoclastogenesis in bone marrow-derived monocytes. This model explains how Th17 T cells increased osteoclast precursor production and osteoclastogenesis was promoted.

METHODS AND MATERIALS

Invertebrates

We used female knockout mice with IL-17 receptor A and TNF receptor (TNFR) p55 knockout mice, as well as wild-type littermates, the animals should be housed in groups of five between the ages of 6 and 8 weeks, in groups of five, under controlled temperatures (22°C / 2°C) and artificial light for 12 hours a day. All mice were housed in positive-pressure cabinets under specific pathogen-free conditions and at all times, sterile food and water are provided.

Infection by bacteria

In tryptic soy broth, Overnight, *Brucella abortus* S2308 was grown. At 37°C, continuous agitation is required. The bacteria were harvested by centrifuging at 6000 rpm for 15 minutes at 4°C and washing twice in 10 mL of PBS. Optical densities at 600 nm were compared. The number of bacteria in stationary-phase cultures was determined using a standard curve obtained in our lab.

Cells and Media

Medium containing 2 mmol/L L-glutamine, 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 g/mL streptomycin was used for all experiments at 37°C in a 5% CO2 atmosphere. Using wild-type mice, thioglycolate-induced peritoneal macrophages were isolated [17]. Following manufacturer's instructions, CD3 positive, CD4 positive, and CD8 positive, a murine spleen was isolated with LTs using kits from BD Biosciences (San Diego, CA). Later, flow cytometry confirmed Pure populations of CD3+, CD4+, and CD8+ and they were >98%, >90%, and >78%. More than 95% of the cells were viable when tested with trypan blue exclusion.

Infection

5×10^5 a well was cultured with peritoneal macrophages from murine peritoneum in complete medium without antibiotics in 24-well plates. The infected cells were infected with *B. abortus* for two hours at different multiplicities of infection (MOI) without antibiotics. For killing remaining extracellular bacteria, cells were washed extensively to remove uninternalized bacteria and infected with antibiotics (gentamicin 100 mg/mL and streptomycin 50 mg/mL) for another 24 hours. For LT stimulation or cytokine determination, harvested Sterilized CSs were filtered through a 0.22-m nitrocellulose filter, and then stored at -70°C. We washed and lysed cells infected with Brucella in parallel at varying intervals after infection with 0.1% (v/v) Triton X-100 in order to monitor intracellular replication of Brucella. Through serial dilutions of bacteria on agar plates with tryptic soy broth, we counted Viable intracellular bacteria (per well). After infection of macrophages in the peritoneum, within 24 hours, the MOIs of bacteria were 25, 9500 x 707, 38,500 x 4949, and 72,500 x 3535.

LT Activation

For 24 hours, Anti-CD3s monoclonal antibody (BD Biosciences) was used to stimulate LT cells (1 x 106 cells per well). Then 0.2 mL of macrophages infected with B. abortus. For determining cytokine secretion in BMM cultures and instigate BMM cultures, LT-derived CS (LTCS) was washed, add to BMM cultures or culture in fresh media for 24 hours.

Osteoclast Formation Assay

A slight modification was made to previous descriptions6 of the induction of osteoclastogenesis in BMMs. IL-17R KO mice, TNFRp mice, or C57BL/6 mice with tumor suppressor gene mutations were cultured recombinant macrophage-specific colony-stimulating factor (M-CSF) is manufactured by R&D Systems in Minneapolis, Minnesota. Cells that were not adherent to the membrane were harvested and cultured with M-CSF at 30ng/mL in 24-well plates for an additional 24 hours. We washed out nonadherent cells and collected adherent cells for use in BMMs. Five million BMMs were plated in Cultured for six days in complete medium containing 30ng/mL for M-CSF in combination with one million T cells stimulated or LTCS (0.2ml). The RANKL (R&D Systems) concentration of 50 ng/mL was used as a positive control for osteoclast formation in BMM cultures. All reagents and culture media were replaced on day 3. TRAP was used to identify osteoclasts in paraformaldehyde-fixed cells. Further, anti-mouse CD51 and anti-mouse CR (BioLeg-end, San Diego) were labeled with phosphatidylethanolamine and fluorescein isothiocyanate, respectively. Multinucleated cells that were positive for either TRAP-CD51 or CR were classified as osteoclasts and were counted microscopically.

Determination of MMP-9 Activity

According to previous reports, using zymography, activity of MMP (matrix metalloproteinase)9 was determined in BMM cultures. On 10% SDS-PAGE gels containing 1 mg/mL gelatin (Sigma Aldrich), differentiated osteoclast ,five times the amount of CSs was loaded onto 10% SDS-PAGE gels as much loading buffer (0.50 mol/L Tris-HCl (pH 7.5) containing 2.5% Triton X-100A, the solution consists of 50 mmol/L

Tris-HCl (pH 7.5) and 2.5% Triton X-100was used to wash the gels after electrophoresis, followed by an addition, 1 mol/L ZnCl2 was added to buffer A for 30 minutes along with 5 mmol/L CaCl2., followed by an incubation period of 48 hours at 37°C of buffer Contains 10 mol/L CaCl2 and 200 mol/L NaCl. During this process, pro-MMP-9 is not proteolytically cleaved, thereby promoting MMP activity. Using 0.5% Coomassie Blue, gels were stained to show gelatin activity. Unstained bands indicate gelatinase activity, and their positions display the enzyme's molecular weight.

Cytokines and RANKL Assays

The levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit of TNF-(alpha), IFN-alpha, and IL-17 in both CS and LTCS.

Pit Formation Assay

We cultured BMMs, M-CSF 30ng/mL and stimulated T cells in complete medium in 96-well dishes containing dentine disks (BD BioCoat Osteologic, San Diego) for six days. The medium and all reagents were changed every day to prevent acidification. NH4OH was used to remove adherent cells after dentine disks were cultured with cells. Resorption lacunae were examined using light microscopy after dentine disks had been rinsed with water.

Neutralization Experiments

An anti-IL-6 or anti-IL-17A antibody or an anti-osteoprotegerin antibody (OPG) were used for neutralization experiments. Each case was controlled with the appropriate isotype. A Brucella-infected cell suspension or the LTCS was pre-incubated for an hour with an antibody (or control receptor isotype) or decoy receptor before it was used.

Modeling Osteoclast Formation in Vivo

As part of the anesthetization of Six- to eight-week-old C57BL/6 mice, ketamine hydrochloride and xylazine (150 mg/kg) were administered intraarticularly to the knee joint followed by intra-articular injections of 1 * The experiments were performed using 106 stimulated T cells, 500 ng LPS from Escherichia coli, or PBS as the control vehicle. Five days after administration, the mouse was sacrificed, and the knee joints were removed. As soon as the knee joints were fixed in 4% paraformaldehyde, they were decalcified in 10% EDTA in 1 mmol/L Tris-HCl (pH 7.3) for 2 weeks at 4°C. A paraffin embedding process was performed on the decalcified specimens.28 Tibial proximal sections were stained for TRAP. Cells with more than three nuclei and TRAP positivity were defined as osteoclasts.

Statistical Analysis

This study used GraphPad Prism 4.0 software (San Diego, CA) for one-way analysis of variance, followed by a Tukey post hoc test. The data are presented as mean x standard deviation.

RESULTS

Bacillus abortus infects macrophages and induces the osteclastogenesis process

In our study, we examined whether T cells activated by B. infantus increased osteoclast production in BMMs. A CS extract was used to stimulate T cells from B. peritonei- Multinucleated cells expressing TRAP, CR, and vitronectin were used to measure osteoclastogenesis in infected peritoneal macrophages. One of the positive controls was RANKL. CS induced osteoclast-like cells in macrophages of *B. abortus*, but not in macrophages of uninfected cells. By evaluating the ability of Brucella-induced osteoclast-like cells to resorb dentine, we hypothesized that *Brucella abortus* infection would induce osteoclasts, which would result in bone loss. Treatment of BMMs with *B. abortus*-activated LT reduced dentine resorption. Dentine resorption was not induced by Cultured LT with CS from uninfected macrophages. Osteoclasts that function as well secrete MMP-9, which degrades organic matrix, MMP-9 can be secreted by them. (Data not shown) Inactivated T cells cannot produce MMP-9. Every marker investigated showed a direct correlation between osteoclastogenesis and MOI used infect macrophages. Also, activated T cell-derived CSs (LTCS) induced osteoclastogenesis. Based on these results, From Brucella-activated LT, functional osteoclastic formation can be induced.

T-cells activated by Brucella mediate osteoclastogenesis

The ability of LT-induced osteoclastogenesis to be induced by CD4+ and CD8+ T cells was evaluated. Macrophages infected with Brucella, CD4+ and CD8+ LTs were activated with BMMs and M-CSF. The TRAP-expressing cell secretes MMP-9 and resorbs dentine. RANKL was a positive control. As demonstrated by MMP-9-secreting and dentin-resorbing TRAP-expressing cells, activated CD4+ T cells generated osteoclasts from BMMs ($P<0.05$). It was found that inactivating LT in the presence of CS from uninfected macrophages did not induce osteoclastogenesis induced by activated CD4+ T cells. The same experimental conditions failed to induce osteoclast-like cells from activated CD8+ LTs. In conclusion, helper T cells induce osteoclastogenesis.

T cells activated by Brucella secrete cytokines that promote osteoclastogenesis

Due to the fact that T cells produce osteoclastogenic cytokines in response to LTCS29, we examined cytokines secreted by LT from Brucella-

infected macrophages treated with CS. After Anti-CD3 activation of LT RANKL and IL-17 were secreted significantly (P - 0.01) by macrophages infected with Brucella. These cytokines were produced by macrophages based on their MOI. CS from uninfected macrophages combined with anti-CD3s treated T cells resulted in copious production of IFN-γ. The number of LTs produced significantly less (P<0.001) when infected with CSs containing increasing amounts of B. abortus. LTs were not infected by or uninfected by CS from macrophages (data not shown). The results of this study effect of T cells on osteoclastogenesis depends on a balance between the two transcription factors RANKL and IL-17, which were both triggered by Brucella-infected macrophages.

Brucella-activated T cells trigger osteoclastogenesis

A major osteoclastogenic cytokine is RANKL29. Thus, we examined Brucella-induced osteoclastogenesis. CS stimulated T cells from B. trachomatis infected macrophages were stimulated with anti-IL-17A antibodies or OPG, a receptor for decoys for RANKL, and TRAP-expressing cells. Brucella-activated T cells were completely blocked by an IL-17A-blocking antibody, but not by an isotype control.

In contrast, RANKL-induced osteoclastogenesis was unaffected by recombinant IL-17. In contrast to RANKL, OPG abrogated IL-17-induced osteoclastogenesis.TRAP-positive cells cannot be induced by RANKL (present in LTCS). The osteoclastogenesis-inhibiting IL-10, might explain this. Osteoclastogenesis was dramatically reduced by culturing Brucella-activated T cells with IL-17R knockout mice. IL-17 failed to induce osteoclasts in IL-17R KO mice, but RANKL induced TRAP-positive cells in BMMs from these mice. IL-17 is required for T cells activated by Brucella induce osteoclastogenesis. An IFN-like molecule secreted by macrophages infected with B. abortus controls LT and osteoclastogenesis

Previously, in our study, we found that B. abortus infection causes various types of cells, including macrophages to release proinflammatory cytokines. IL-6 was prominent among these cytokines. Due to the pivotal role that IL-6 played in the induction of Th17 cells, the role of LT in Brucella-activated osteoclastogenesis was investigated. In order to activate LT, Brucella macrophage CSs were activated by anti-IL-6 antibody and a control antibody of its isotype. For osteoclastogenesis, we co-cultured Brucella activated LT with BMMs and M-CSF in presence of cells positive for TRAP. Neutralizing IL-6, LTCS was less likely to produce IL-17 and osteoclastogenesis was induced by Brucella-infected macrophages activated by CS.Neither IL-17 production by T cells nor osteoclastogenesis was affected by isotype control. LT were found to produce pro-osteoclastogenic

Osteoclastogenesis is mediated mainly by osteoclast precursors induced by IL-17

Osteoimmunologically, RANKL is activated by IL-17, releasing proinflammatory cytokines into precursors and supporting cells of osteoclasts. In pathological conditions, the most important osteoclastogenic molecule is TNF, not RANKL. M-CSF was added to BMM,based on the results obtained from TNFRp55 KO mice that were stimulated with CS released by B. aquatus-infected macrophages, and TRAP expression was assessed. Control BMMs were C57BL/6. In BMMs of Brucella-infected macrophages pretreated with TNFRp55 KO mice at low MOI, LTs release IL-17, but these cells do not induce osteoclastogenesis, or significantly reduce osteoclastogenesis compared to wild-type mice. TNFRp55 KO BMMs can induce osteoclastogenesis, in macrophages infected with Brucella at 100 MOI, CS is produced pretreatment with wild-type LT. Treatment of wild-type LTs with macrophage CSs at MOI 100 induced IL-6 and IL-1 expression in TNFRp55 KO mice. TNFRp55 KO BMMs did not induce TRAP-positive cells Osteoclast precursors produced the proinflammatory triad of cytokines, among them, TNF-α induced osteoclastogenesis.

Representative of two experiments conducted.

Osteoclasts are induced in mice's tibiae by Brucella-activated T cells

As a final step, Brucella-activated T cells were injected into C57BL/6 wild-type mice in order to test the relevance of our hypothesis in vivo. *E. coli* LPS was used as a positive control, and phosphate buffer solution was used as a negative control. On histological sections taken five days after sacrifice, knees were dissected and proximal tibiae stained for TRAP. As defined by the tibias of animals treated with Brucella-infected macrophage CS injected with LT revealed the presence of multinucleated cells positive for TRAP showed extensive osteoclastogenesis. In contrast, osteoclastogenesis was not observed in inoculated animals treated with macrophage CS. E. coli LPS induces osteoclastogenesis extensively, as does PBS. As a result, activated the *B.anortus*. T-cells in bone tissue induced a multinucleated TRAP-positive osteoclast response in the bone tissue

Figure:1 BMMs, cultured with M-CSF and anti-CD3-activated T cells stimulated by CS from B. abortus–infected macrophages, exhibit osteoclastogenesis. RANKL serves as a positive control. After 6 days, osteoclastogenesis is assessed by multinucleated TRAP, vitronectin receptor (CD51), and CR-expressing cells. Functional osteoclast-like cells demonstrate dentine resorption and secrete MMP-9, as confirmed by zymography. Data represent the mean ± SEM of duplicates.

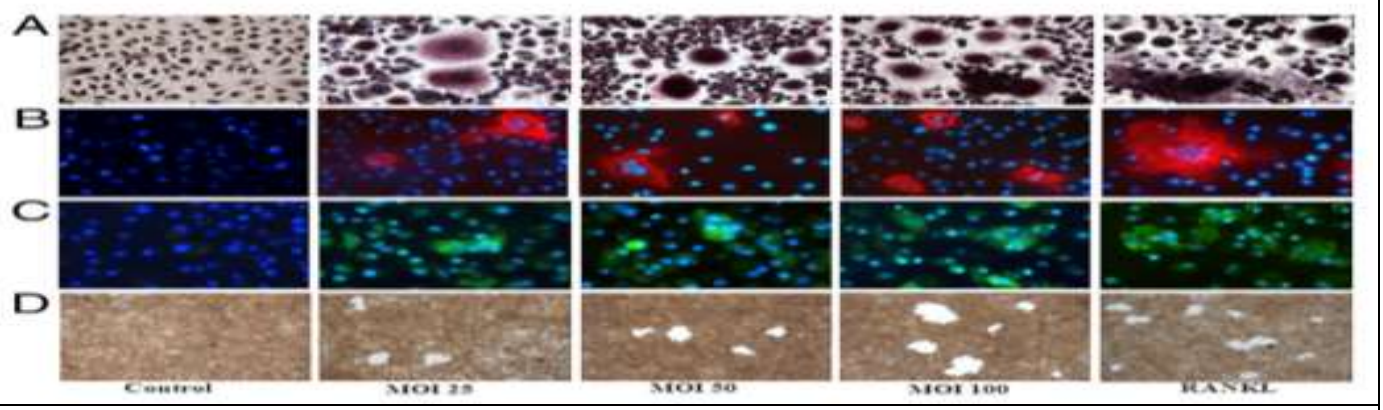
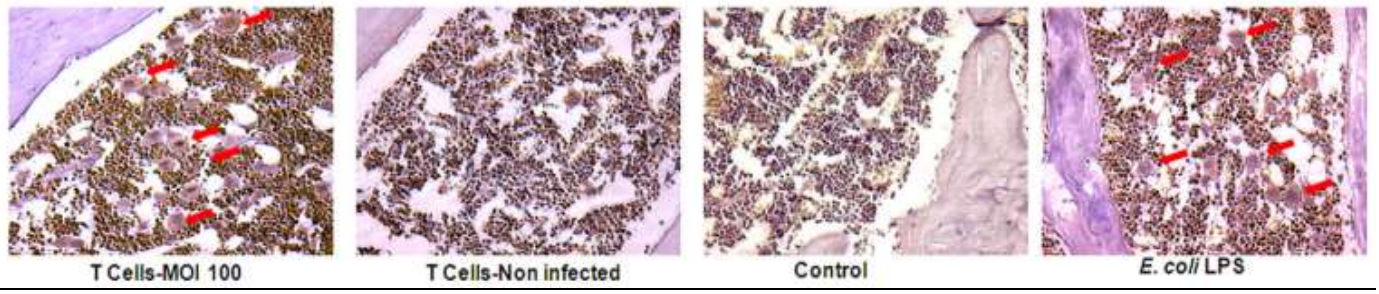


Figure: 2 Brucella-activated T cells induce osteoclastogenesis by generating osteoclast-like cells in the tibiae of mice. After 5 days, longitudinal sections of mouse tibiae inoculated with Brucella-activated T cells reveal TRAP-positive multinucleated cells (arrows), similar to those observed in animals injected with E. coli LPS. Conversely, LTs treated with CS from uninfected (control) peritoneal macrophages do not exhibit TRAP staining. The presented data are representative of two experiments conducted.



DISCUSSION

Osteoimmunology studies reveal there is a close relationship between the immune system and the skeletal system. Studies of arthritis and other inflammatory conditions have found that T cells and osteoclasts have received significant attention. It is essential to determine whether and how T cells contribute to enhanced osteo-oclastic bone resorption in osteoarticular brucellosis because infiltration of T cells into the bone and joints is a hallmark pathological finding of osteoarticular brucellosis. In this study, in this study, we examine how pre-activated T cells, stimulated by B. abortionis macrophages, promote osteoclastogenesis. MMP-9-active multinucleated cells with TRAP, CR, and vitronectin receptors corroborate osteoclast development phenotypically, and these cells can induce dentine resorption functionally. In addition, B. abortion-stimulated T cells in mice's tibiae induced Multinucleated osteoclasts positive for TRAP, further demonstrating the role of LTs in Osteoclastogenesis following infection with *B. abortus*.

LTs that induced osteoclastogenesis included helper cells. Despite preactivation with anti-CD3, B. embryonus macrophages promote osteoclastogenesis by secreting osteoclastogenesis-promoting cytokines. The results demonstrate that IL-17 contributes to osteoclastogenesis induced by Brucella-activated T cells, despite RANKL's role in osteoclast differentiation [16]. There are several reasons why B. infantus-activated T cells cannot induce osteoclastogenesis without IL-17. T-cell–induced RANKL may not be the primary factor in osteoclastogenesis, despite Th17 cells' ability to secrete RANKL.

In contrast, mesenchymal cells express RANKL, which initiates osteoclastogenesis. Additionally, IL-10 was produced by T cells activated by Brucella, IL-17, and RANK, a cytokine that inhibits osteoclastogenesis. There was an increase in T cell recruitment in collagen-induced arthritis, which produced IL-17. This, along Cytokines that promote inflammation, is a characteristic of B. It has also been shown that B. can induce abortion [17]. Even so, IL-17 plays a significant role in osteoclastogenesis during pregnancy. It has been shown that Th17 cells directly contribute to osteoclastogenesis in only a few studies. However, Mesenchymal support cells (fibroblasts and osteoblasts) are absent. Th17 cells cannot induce osteoclastogenesis.Osteoclastogenesis is primarily induced by IL-17 inducing pro-inflammatory cytokines. It releases prostaglandins, TNF-, IL-1, and IL-6 during osteoclastogenesis [18]. The osteoclast precursors are

directly stimulated by RANKL and M-CSF, which act in synergy with RANKL in driving osteoclastogenesis. Osteoclastogenesis is driven by several different mechanisms including the increase of osteoclast precursors and RANKL expression in osteoclastogenic supporting cells in pathological conditions.

LT produced pro-osteoclastogenic IL-17 when exposed to Inflammatory mediators produced by B. Abortus infection, including IL-6. IL-6 and transforming growth factor- play an important role in Th17 differentiation. In addition to these results, previous research has shown that macrophages infected with B. abortus do not produce transforming growth factors suggest that IL-6 contributes to IL-17's osteoblastogenic effect. Despite its role at the co-adjuvant stage of Th17 differentiation induced by Brucella infection in Macrophages infected with abortus.

CONCLUSION

In order to establish Brucella immunity, the immune system is stimulated by the infection, resulting in a pro-inflammatory response that favors the differentiation of T cells into Th1 cells. It is unclear how Immunopathological characteristics of T-cell responses induced by Brucella. Bone loss due to B. canis. The B. induced osteoclastogenesis. Our findings complement those of previous studies observation of the role of IFN-γ in Brucella infection. Upon local inflammation, Brucella-specific Pathogenic Th17 cells develop from Th1 cells. In the near future, understanding what regulates the CD4+ T cells specific for Brucella will be produced, need to be stable and plastic to better combat infection and control osteoarticular brucellosis.

REFERENCES

1. Aydin M, Fuat Yapar A, Savas L, Reyhan M, Pourbagher A, Turunc TY, Ziya Demiroglu Y, Yologlu NA, Aktas A. (2005). Scintigraphic findings in osteoarticular brucellosis. Nucl Med Commun, 26, 639 – 647
2. Colmenero JD, Ruiz-Mesa JD, Plata A, Bermudez P, Martin-Rico P, Queipo-Ortuno MI, Reguera JM. (2008). Clinical findings, therapeutic approach, and outcome of brucellar vertebral osteomyelitis. Clin Infect Dis, 46, 426 – 433
3. Madkour MM. (2001). Osteoarticular brucellosis. Madkour's Brucellosis, ed 2. Edited by MM Madkour. Berlin, Springer-Verlag, pp 74 – 84
4. Madkour MM. (2001). Bone and joint imaging. Madkour's Brucellosis, ed 2. Edited by MM Madkour. Berlin, Springer-Verlag, pp 90 –132
5. Young EJ. (1989). Clinical manifestations of human brucellosis. Brucellosis: Clinical and Laboratory Aspects. Edited by EJ Young, MJ Corbel. Boca Raton, CRC Press, pp 97–126
6. Delpino MV, Barrionuevo P, Macedo GC, Oliveira SC, Genaro SD, Scian R, Miraglia MC, Fossati CA, Baldi PC, Giambartolomei GH. (2012). Macrophage-elicited osteoclastogenesis in response to Brucella abortus infection requires TLR2/MyD88-dependent TNF-alpha pro- duction. J Leukoc Biol, 91, 285–298
7. Haynes DR. (2004). Bone lysis and inflammation. Inflamm Res, 53, 596 – 600
8. Merkel KD, Erdmann JM, McHugh KP, Abu-Am Y, Ross FP, Teitelbaum SL. (1999). Tumor necrosis factor-alpha mediates orthopedic implant osteolysis. Am J Pathol, 154, 203–210
9. Nair SP, Meghji S, Wilson M, Reddi K, White P, Henderson B. (1996). Bacterially induced bone destruction: mechanisms and misconceptions. Infect Immun, 64, 2371–2380
10. Wei S, Kitaura H, Zhou P, Ross FP, Teitelbaum SL. (2005). IL-1 mediates TNF-induced osteoclastogenesis. J Clin Invest, 115, 282–290
11. Kotake S, Sato K, Kim KJ, Takahashi N, Udagawa N, Nakamura I, Yamaguchi A, Kishimoto T, Suda T, Kashiwazaki S. (1996). Interleukin-6 and soluble interleukin-6 receptors in the synovial fluids from rheumatoid arthritis patients are responsible for osteoclast-like cell formation. J Bone Miner Res, 11, 88 –95
12. Revell PA, Mayston V, Lalor P, Mapp P. (1988). The synovial membrane in osteoarthritis: a histological study including the characterisation of the cellular infiltrate present in inflammatory osteoarthritis using monoclonal antibodies. Ann Rheum Dis, 47, 300 –307
13. Smith MD, O'Donnell J, Highton J, Palmer DG, Rozenbilds M, Roberts-Thomson PJ. (1992). Immunohistochemical analysis of synovial mem- branes from inflammatory and non-inflammatory arthritides: scarcity of CD5 positive B cells and IL2 receptor bearing T cells. Pathology, 24, 19 –26
14. Chu CQ, Field M, Allard S, Abney E, Feldmann M, Maini RN. (1992). Detection of cytokines at the cartilage/pannus junction in patients with rheuma- toid arthritis: implications for the role of cytokines in cartilage destruction and repair. Br J Rheumatol, 31, 653– 661
15. Cenci S, Toraldo G, Weitzmann MN, Roggia C, Gao Y, Qian WP, Sierra O, Pacifici R. (2003). Estrogen deficiency induces bone loss by increasing T cell proliferation and lifespan through IFN-gamma-induced class II transactivator. Proc Natl Acad Sci U S A, 100, 10405–10410
16. Cenci S, Weitzmann MN, Roggia C, Namba N, Novack D, Woodring J, Pacifici R. (2000). Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-alpha. J Clin Invest, 106, 1229 –1237

17. Roggia C, Gao Y, Cenci S, Weitzmann MN, Toraldo G, Isaia G, Pacifici R. (2001). Up-regulation of TNF-producing T cells in the bone marrow: a key mechanism by which estrogen deficiency induces bone loss in vivo. Proc Natl Acad Sci U S A, 98, 13960 –13965
18. Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, Capparelli C, Li J, Elliott R, McCabe S, Wong T, Campagnuolo G, Moran E, Bogoch ER, Van G, Nguyen LT, Ohashi PS, Lacey DL, Fish E, Boyle WJ, Penninger JM. (1999). Activated T cells regulate bone loss and joint destruc- tion in adjuvant arthritis through osteoprotegerin ligand. Nature, 402, 304 –309