META-ANALYSIS OF OSTEOIMMUNOLOGY: INTERACTIONS OF THE BONE AND IMMUNE SYSTEM

Dissertation submitted in partial fulfillment for the requirement of the degree of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

By

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UNDER THE GUIDANCE OF
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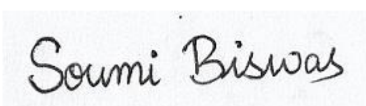
TABLE OF CONTENTS

DECLARATION

I hereby declare that work presented in this report entitled **"Meta-analysis of osteoimmnunology: Interactions of the bone and immune system "** in partial fulfilment of the requirements for the award of degree in Bachelor of Technology in the Department of Biotechnology and Bioinformatics **from Jaypee University of Information Technology Waknaghat, Solan, H.P.** is an authentic record of my own work carried out under the supervision of **Dr. Rahul Shrivastava**, Associate Professor in the Department of Biotechnology and Bioinformatics. I have not submitted this work elsewhere for any other degree or diploma.



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SUPERVISOR'S CERTIFICATE

This is to certify that the review of literature reported in the B.Tech thesis entitled **"Meta-analysis of osteoimmnunology: Interactions of the bone And immune system",** submitted by **Soumi Biswas (171811) at Jaypee University of Information Technology, Waknaghat, India,** is a bonafide record of her original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

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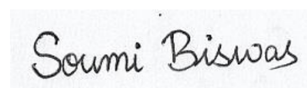

Soumi Biswas (171811)

LIST OF FIGURES

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ABSTRACT

Recently, extensive reciprocal interactions between the immune and skeletal systems demons
trated, to the rise of a new interdisciplinary research field, named “osteoimmunology”, that is fo
cused on the understanding of the crosstalk between the bone and immune systems. In brief, t
his field investigates the nexus between the immune system impacting the bone turnover in pa
thological and physiological conditions through the immune-skeletal interface. The researchers
have gained better understanding of the interactions between bone cells and immune syst
em, and opened horizons in bone remodeling, wherein the bone formation and resorption coexi
st in a dynamic equilibrium were under strict immunological control. Nevertheless, bone and im
mune system are functionally integrated through the complex homeostatic networks and hence,
provides a framework for obtaining new insights for the discovery of novel treatments fo
r diseases related to both systems. Therefore, osteoimmunology definitely appears as both
an interdisciplinary researchand clinical field which allows new pathogenetic and clinical i
nterpretations of wellknown and common diseases, such as osteoporosis, rheumatoid arthrit
is, periodontitis, etc. The field of interest is constantly expanding, thus enriching with an i
ncreasing number of translational implications, in clinical practice and even in various bra
nches of medicine.

CHAPTER 1: INTRODUCTION

1.1 What isosteoimmunology?

Osteoimmunology is an emerging field of research that explains the relationship between the immune processes and the bone metabolism of various inflammatory bone diseases. The mechanisms governing the osteoblast and osteoclast are critical for the understanding of the health and disea se of the skeletal system. The field investigates interactions between skeleton and immune sys tem.

The term osteoimmunology was first time coined by Arron and Choi, to describe the phe nomenon of T-
cellmediated regulation of the osteoclasts. Also, most of the research work carried out in t his field are quite recent and are mainly focused on the influence of the immune system on osteoclast physiology. As a matter of fact, the bone cells share a common origin wit h immune cells, since they both arise from bone marrow hematopoietic stem cells.

1.2 Importance ofosteoimmunology:

The immune system is an organization of cells and molecules with specialized roles in defendin g against pathogenic bacteria (extra and intracellular), viruses (intracellular), fungi, protozoa oth er parasites and also to protect us from neoplastic cells.When a pathogen enters the human body for the very first time, it immediately encounters cells of the innate immune system that are con stantly patrolling for foreign invaders. The macrophages, neutrophils and dendritic cells acts as t he defense mechanism, which engulf and destroy the foreign pathogens and the infected body c ells as well. The guard cells then break down the material they have ingested and display sampl es of the intruder’s components known as antigens so that members of the adaptive immune syst em, T and B cells, can become familiar with the pathogen’s appearance. At the same time, the a ntigenthe killer T cells destroy the cells colonized by the invading microbes while the B cells release antibodymolecules. It takes time for the interactions to happen with antigen presenting cells to create theseB and Tcells, buta subset remains in thebodyas the"memory"cells. There is a close relationship between the immune and bone systems. Res earch into the bone destruction associated with inflammatory diseases such as osteoporosis, rheumatoid arthritis, periodontal disease, osteoarthritis, Paget's disease, multiple myeloma an d metastatic bone tumors highlights the importance of the inter play of the immune and sk eletal systems. The crosstalk between these systems has led to the emergence of an interdi sciplinary field called osteoimmunology. Although, osteoimmunology initially started with the s tudy of the immune regulation of osteoclasts but, its scope has now been extended to encompass a wid e range of cellular and molecular interactions, including those between osteoclasts and osteoblast s, osteoclasts and lymphocytes, and hematopoietic cells and osteoblast.

At the same time, various factors produced during the immune responses are also significantly capable of effecting the bone regulation and metabolism. Therefore, the two systems needs to be understood thereby, to integrate and operate in the context of the osteoimmune system, allo wing a heuristic concept which aims to provide a scientific basis for the discovery of novel tre atments for diseases related to both systems but also, a framework for obtaining new insights by basic research too.

1.3 What is bonebiology?

Bone is a key element of the skeletal system which evidently supports locomotor activity in vertebrates, but is simultaneously a multifunctional organ that deposits and maintains the metabolic homeostasis of minerals such as calcium and phosphate. In addition, bone arr ow harbors mature immune cells and hematopoietic stem cells, including B cells, a small number of T cells and macrophages. The host-defense mechanism of immune system maintains byelements including virus, cancer and bacteria. The immune system starts from the plants and primitive animals and therefore, forms the basis for complex systems of innate and adaptive i mmunities found especially in vertebrates. However, the highly developed vertebrate immune system requires both the tissues and functionally specialized immune cells for the generation a nd maintenance of cells, such as the thymus, spleen, lymph node and bone marrow.

CHAPTER 2: BONE CELLS AND THE IMMUNE SYSTEM

2.1 The origin of bone cell:

i. **Osteoclasts:**

Osteoclasts are multinucleated giant cells that form from the fusion of mononuclear precursor cells. Mature osteoclasts are unique in their capacity to efficiently resorb bone and contain a variety of specific cell structures that facilitate this process. Th e origin of the osteoclast precursor cell has been well studied. Initial work demonstr ated that osteoclasts share many characteristics with macrophages. Although, osteocl asts and macrophages appear to express some common antigens, there are also clear differences in the expression of surface antigens that separate these two cell types. Mononuclear cells, which can differentiate into osteoclastlike cells (OCL) in a var iety of in vitro culture systems, are present in the bone marrow and the periphera l blood. The availability of multiple antibodies recognizing cell surface molecules, which are expressed on hematopoietic cells, has allowed the identification of bone marrow peripheral blood and spleen cell populations that can form OCL in vitro.

ii. **Osteoblasts:** Osteoblasts are derived from a mesenchymal progenitor cell that is multipo tential and also can differentiate into marrow stromal cells and adipocytes. Although, the signals that regulate the decision of mesenchymal progenitor cells to form osteoblasts ar e incompletely understood. However, a number of critical paracrine signals and cell auto nomous transcription factors have been identified. These include the transcription factors Runx2 and osterix, which when absent prevent osteoblast formation, and the bone morp hogenic protein (BMP) family, which initiates the signals for osteoblast differentiation. Most recently, it was found that Wnt signaling pathways are involved in the decision of t he mesenchymal progenitor cell to become either an adipocyte or an osteoblast. As matri x calcifies under the influence of the osteoblast produced enzyme.Osteocytestheninterco nnect with each other with the cells present at bone surface via cellular projections, which called dendriticprocesses which reside in the form of channels in the mineralized bone, termedcanaliculi.

iii. **Osteocytes:** Osteocytes produces the Receptor Activator of Nuclear factor Kappa B ligand (RANKL) in the bone, therefore since this cytokine is important for lymph ocyte development as cell type could infImmune system. Indeed, it has been demonstrated t hat RANKL arising from osteocytes contributes to the bone loss and increased osteoclastog enesis observed in estrogen deficient conditions. Subsequently, particular deletion of the R ankl gene in the osteocytes prevents increase in B cell formation.

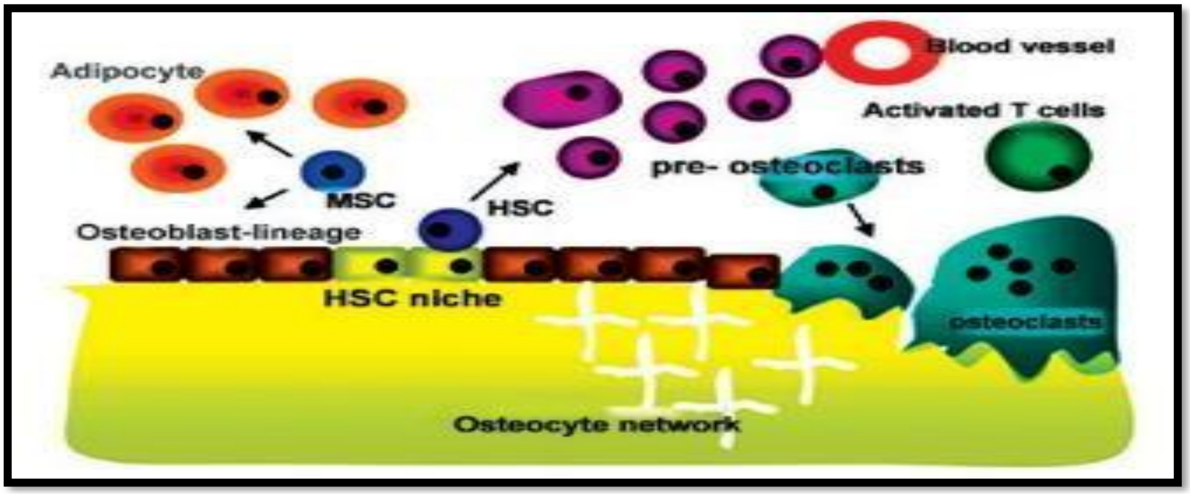

Figure 1: The bone environment

2.2 Immune cells in bone physiology:

Bone cells influences the immune system, and employs theimmune factors for their physiologic fu nction and vice-versa, as described below:

1. **T-Cells:** T-cells are component of adaptive immunity. These cells also play a role in osteoimmunology. Tcells are not all created equal, and in this group , we can find CD4+ T- helpers, cytotoxic CD8+ T-cells, further subcategorized in Th17, Th2, Th1, and T-reg cells, which plays a in role in preventing excessive or improper (e.g.self-directed)immuneresponse.The links between T-cells and bone biology are numerous, essentially all the subtypes of T-cells are able to influence bone cells (mostly osteoclasts). The expression of a cytokine signature: IL-17A, IL-17F, IL-22, IL-26, and IFN-γ. These cells can induce Macrophage Colony-Stimulating Factor (M-CSF) and RANKL expression in osteoblasts and stromal cells, produceRANKL and TNF-α, increasing RANK expression in osteoclast precursors. These features make them potent osteocla stogenesis inducers, which have been already described as players in human bone diseases, such as RA and multiple myeloma.
2. **Dendritic Cells:** Dendritic Cells (DCs) are the antigenpresenting cells that important role of

directing cellmediated immunity toward the right targets, as quickly as possible and avoi dimmunity. Their role in bone biology has in fact been historically thought as mostly ind irect, through Tcells. Dendritic cells also regulate subtype balance through cytokine sign aling and their activity. An interesting concept that could be important in Rheumatoid Ar thritis (RA) is that DCs transdifferentiate into osteoclasts through RANKL stimulation a nd MCSF. Since DCs are numerous in number hence, they could wellcontribute to the os teolytic disease like RA. However, DCs has not been investigated in human studies.

3. **Neutrophils:** Neutrophils play a pivotal role in bone biology, and in particular, in the inflammation-induced bone loss. In fact, neutrophils are the first cell type migrating to damage sites, including bone, where they secrete cytokines, many chemokines and small molecules, which acts as immunomodulatory factors. However, the absence of neutrophils is more damaging to the bone tissue since, it results in local IL-17-driven inflammatory bone loss eventually. The activated neutrophils express RANKL in the inflammatory site, and if that site is the synovium, they can also participate in osteoclastogenesis, thereby increasing the RA-related osteolysis. However, the role of neutrophils in osteoimmunology is not cut-and dried, and that the activated neutrophils are surely the osteoclastogenesis inducers having effects, both directly andindirectly.
4. **NaturalKiller(NK)Cells:** NaturalKiller(NK)cells helps intheregulatingthe boneenvironment. Theyareinvolved in bone destruction induced by RA, and in osteoblast cell death, thereby making these cells a potential therapeutic target to reduce RA-induced bonedestruction.
5. **OsteomacsandBoneMarrowMacrophages:** Boneandbone marrow present with resident macrophages, which include bone marrow macrophagesand osteal macrophages. The latter, also known as osteomacs, are TRAcP negative and F4/80 positive, located close to the bone surface and are versatile cells, able to regulate bone mass and become osteoclasts, and also, participate actively in the homeostasis of the immune system. Inflammation and Inflammatory Factors Several cells of the immune system (T and B cells, NK cells, monocyte/macrophage and dendritic cells) produce InterFeroN (IFN)-γ, which has a pivotal role in innate and adaptive immune responses as well as in the regulation ofinflammation.

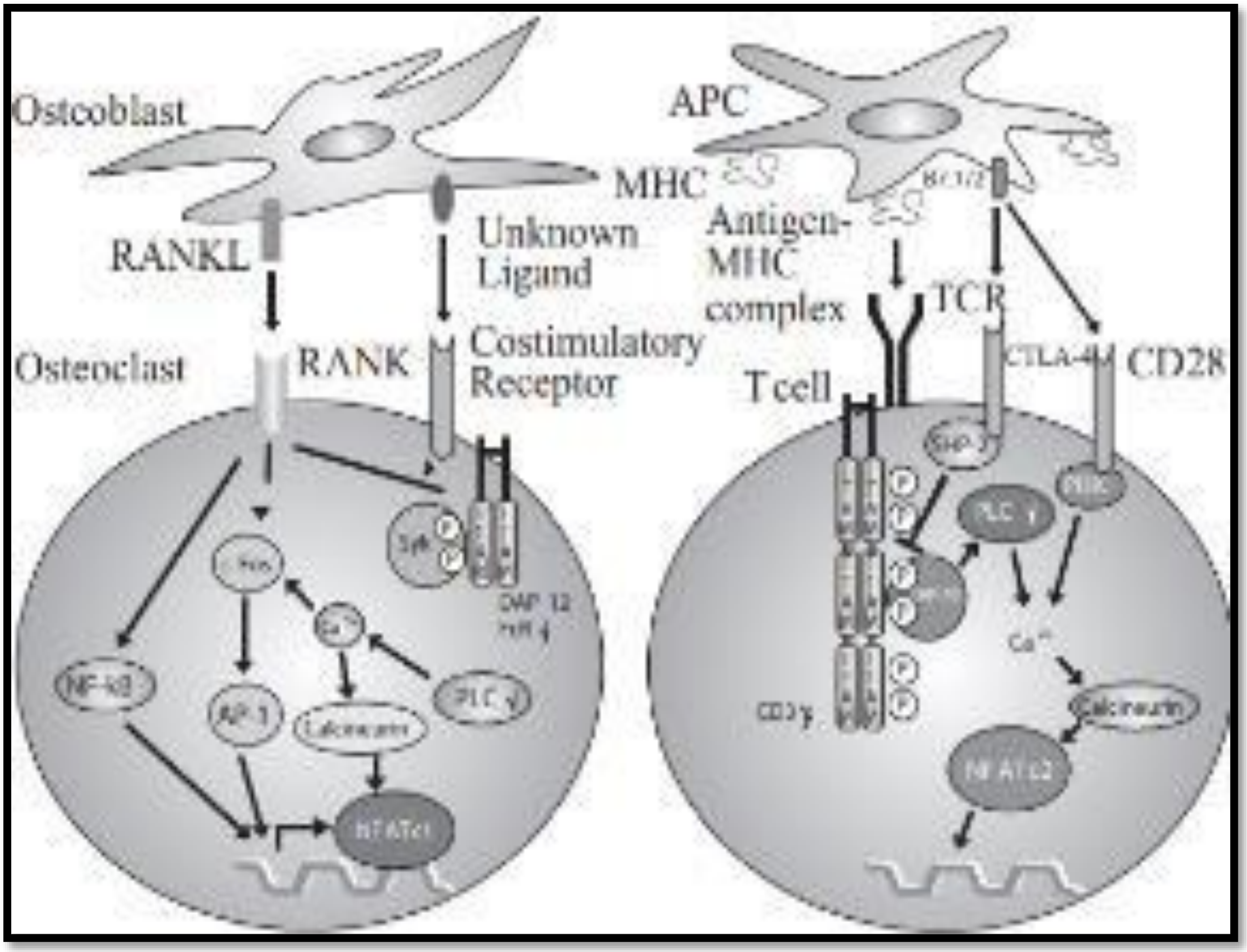

Figure 2: The immune interactions

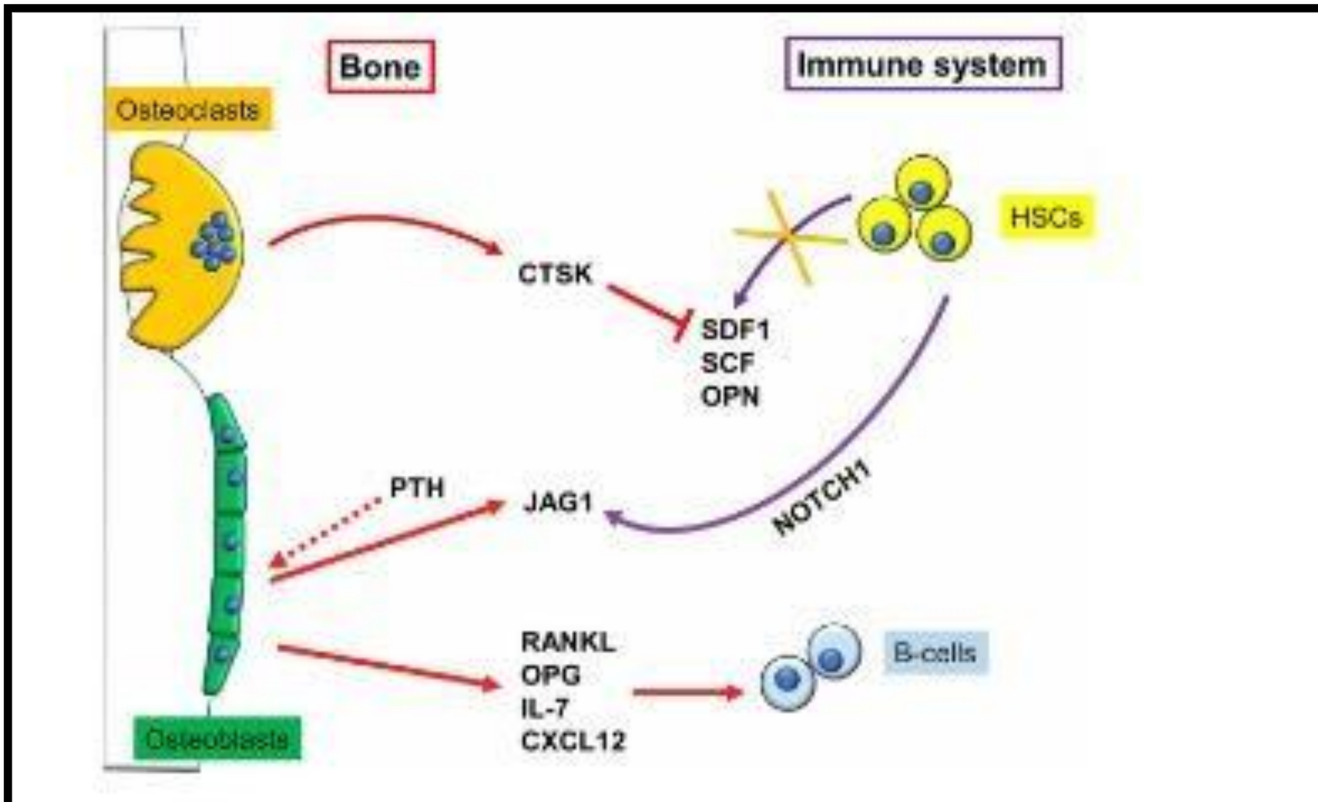

Figure 3: The regulation of the immune cells by bone cells. Osteoclasts reduce hematopoietic stem cells (HSCs) by secretion of the cathepsin K (CTSK) thatis involved in degrading the stemcellfactor(SCF),stromal cell- derivedfactor(SDF),andosteopontin(OPN)deprivingtheboneniche ofthe HSC-bindingsites.After stimulation, the osteoblasts alongwiththe help of pro- osteoblastogenic factors such as Jagged1 (Jag 1), intermittent parathyroid hormone (PTH), binds with the NOTCH1 on HSCs, andallowBcells interactinseveralways.

CHAPTER 3: THE BONE REMODELLING

3.1 Bone remodellingprocess:

Bone remodelling **is** a coordinated process between formation and degradation of bone, r espectively managed by osteoblasts (OBs) and osteoclasts (OCs), ensuring bone homeosta sis. During bone remodeling, damaged and/or aged bone is being replaced by an equivale nt amount of new bone to maintain normal bone mass and quality. Bone resorption is ti ghtly coupled with subsequent bone formation in a remodeling process to achieve this. T he process of linking resorption to formation is called “coupling”, and it has received co nsiderable attention in bone research. Bone remodeling is carried out in three sequential phases: the “initiation” of bone resorption by osteoclasts, the “transition” from resorption t o new bone formation (also well known as “reversal” period), and “bone formation”.

Bone was viewed as a static tissue, “scaffolding” for all the other organs, whereas, bone is a organ that is dynamic, that undergoes continuous cycles of modeling during growth and remodeling during adulthood, guaranteeing proper bone shape. Bone modeling and remodeling are guaranteed by the mechanism of:osteoblasts,whichdeposebone,osteoclasts,whichresorbbone,andosteocytes,which are former osteoblasts buried in bone matrix, controlling bone mechanophysiology, and able to resorb and depose bone. The process of bone remodeling is categorized into four phasesnamely:

1. Latent phase: Osteocytes activate the bone-lining cells followed by a stimulus, whereby initiation of differentiation of osteoclast and exposition to the bonesurface;
2. Activation phase: Osteoclasts then helps in resorption of the bone remained by the bone-lining cells and then detach themselves from the bone and undergoapoptosis;
3. Reverse phase: Macrophage-like cells migrates to the lacuna and cleans thedebrisleftbythereversecells and osteoclasts,andsecretesthatsummonsthe osteoblast in resorptionlacuna;
4. Formation phase: this is the lengthiest phase in the bone remodeling process, lasting up to 6-8 months, wherein,theosteoblastsoccupythelacuna for resorptionandfillthemwiththeorganicosteoid

matrix and finally mineralizes. In this end phase, osteoblasts undergoes apoptosis, or may embed themselves in the bone matrix they've produced, forming osteocytes.

Bone modeling and remodeling are identical processes sharing same mechanisms. In modeling happens during growth and fracture repair, and guaranteeing the mass accrual, while remodeling happens in adulthood and does not change the bone mass, but keeps mechanical property at physiological levels by renewal process.

The coupling mechanism hypothesis was first proposed by Baylinkin 1981, basedonthesoluble"couplingfactor"releasedfromthebonematrixduringbone resorption that stimulated bone formation in organ culture. Nevertheless, the evidence from mouse genetics and human confirms this hypothesis that, patients with osteopetrosis due to the absence of osteoclasts had a reduced bone resorption as well as the reduced bone formation; and osteoclasts, suggesting that coupling factors are provided by osteoclasts themselves but, they don't resorbthe bone and are released from the matrix during the process of the resorption of bone.

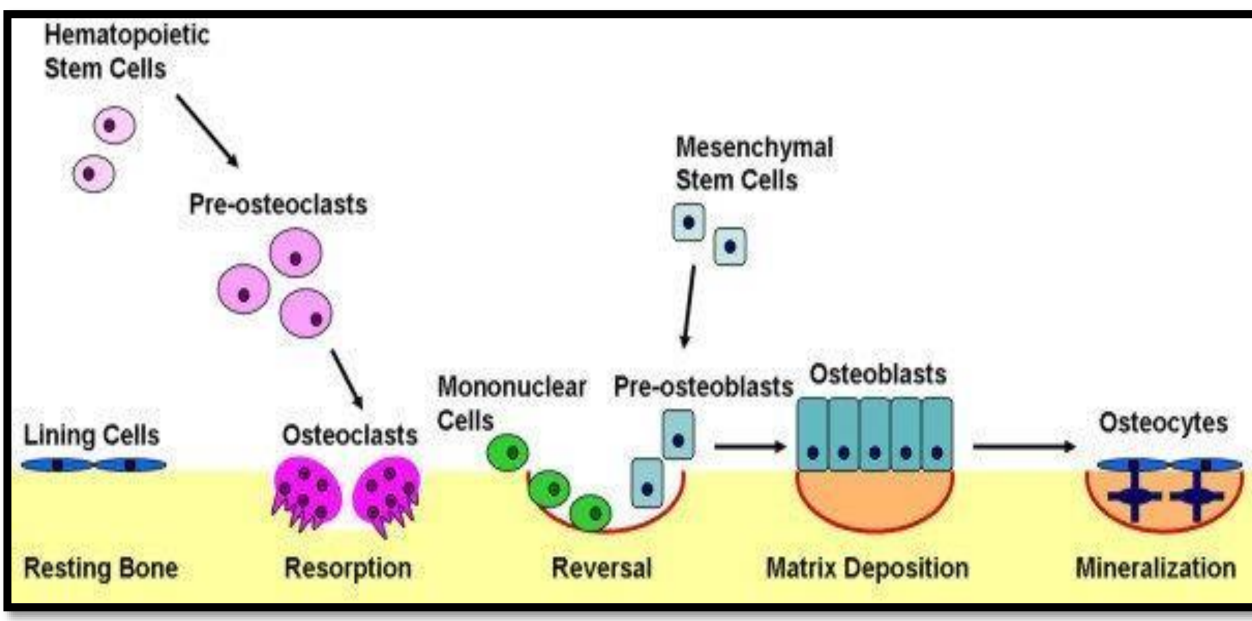

Figure 4: The bone remodelling cycle

3.2 Cellular and molecular mechanisms in boneremodelling:

1. The Basic MulticellularUnit

Bone is a dynamic tissue formed by a protein and mineral salt matrix in which a re embedded the bone cells, osteocytes (OCy), osteoblasts (OB) and osteoclasts (O C). Subsequently, other cells taking part in bone composition, including cartilage, stromal, mesenchymal and hematopoietic stem cells, are all linked by a dense net work of signals. Antagonistic signaling between skeletal stem cellderived subsets is a key mechanism in skeletal subset lineage commitment. Bone tissue undergoes c ontinuous adaptation during lifetime to preserve the structure of the skeleton and t o control the mineral homeostasis. Bone turnover requires two coordinated processe s: bone formation, driven by OB and bone resorption, mediated by OC.

OCy, by a complex network of channels, transmit micro-traumatic and mechanical signals for activation of repairing. They exert a fundamental role in the control of OB and OC functions. The OCy synthesize the mineral component, and the bone matrix proteins, which helps in determination of the quality of the bone.There are multiple subpopulations of perisinusoidal mesenchymal stem/progenitor cells (MSPCs), that have specific relationships with the different kinds of niche, i.e. the s urrounding microenvironment in which the selfrenewal and multilineage stem cells proli ferate and differentiate.

The stem cells that repair and maintain the postnatal skeleton is an osteochondroreticular (OCR) stem cell that generate reticular marrow stromal cells ,OB, chondrocytes, but not adipocytes. They are characterized by the expression of the bone morphogenetic protei n (BMP) antagonist gremlin 1 (Grem 1). The perisinusoidal MSC population also contai ns NesGFP, leptin receptor (Lepr)cre and CD146 expressing cells with osteogenic and a dipogenic potential.

The osteoblast precursor cells (OBP) after increasing the osteopontin receptor (CD44) an d the receptor for stromal cellderived factor 1 SDF1 (CXCR4) expression, migrate an

OC are the multinucleated myeloid cells, specialized in the removal of mineralized bone matrix by the production of catepsin k and lysosomal enzymes, such as tart rateresistant acid phosphatase (TRAP), against a selective inhibitor (odanacatib) wh ich has been recently synthesized to be employed in the osteoporotic patients. The y're derived from a bone marrow precursor which subsequently, gives rise to prof essional antigen presenting cells (APC), i.e., macrophages and dendritic cells. OC may be therefore maybe considered as specialized immune cells. Also, OB, OCy a nd OC communicate with each other continuously as to optimize the bone quality.

2. The ReceptorNetwork

The binding of RANK receptor on OC and their precursors by its ligand RANKL, expre ssed by OB and stromal cells, is the main activation signal for bone resorption. The OB derived MCSF links to its receptor cfms on the surface of osteoclast cell precursors (OC P), enabling the RANK/RANKL signal. Osteoprotegerin (OPG) inhibits osteoclastogene sis by receptor of RANKL, thus preventing bone resorption. RANK receptor on OC, throug h the facilitation of the tumor-necrosis-factor- receptor-associated factor 6 (TRAF6), bound to bind with the cytoplasmic tail, activates NF-kB and other transcription factors, such as MAPKs, cfos, activator protein 1 (AP1), up to nuclear factor of activated T cells (NFATc1), the hub of various signaling pathwa ys. Simultaneously, the activation of RANK induce the phosphorylation that are associ ated with the adaptor proteins, like the immunoreceptor tyrosine-based activation motif (ITAM) and Fcreceptor common gamma (FcRγ). Many other rec eptor pathways interact with RANK, some costimulators and amplificators, others inhibit ors and modulators, and many of these are shared by immune cells. An inhibitor receptor system for RANK signal is ephrin (Eph) B2/B4. EphB2 receptor on OC, stimulated by EphB4 ligand on OB, inhibits the OC differentiation blocking c-fos and the NFATc1transcriptional cascade.

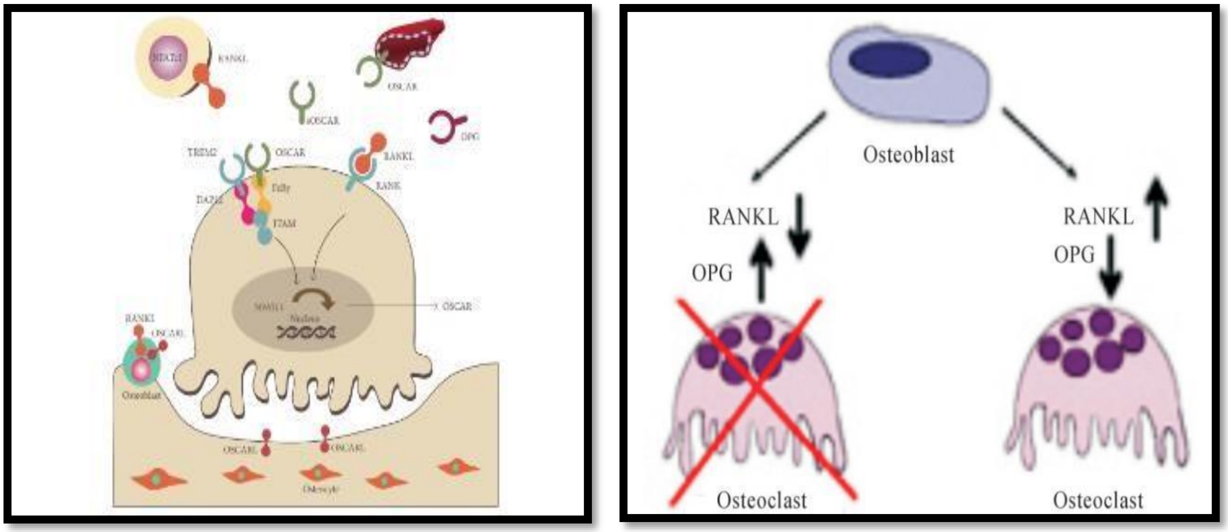

Figure 5 & 6: RANKL-RANK-OPG axis and ITAM pathway in osteoimmunology

3.3 Essential role of RANKL in bone metabolism:

RANKL is a membranebound molecule, and the soluble form is produced by a shedding pro cess that is mediated by proteolytic cleavage by metalloproteinases such as MMP-14. Both the soluble and membrane bound forms of RANKL function as agonistic ligands for RANK. However, the membranebound RANKL is more efficient than its soluble form. RANKL serves as a surviva l factor for osteoclast lineage and chemotactic cells, but it's unclear how the membranebound and soluble forms specifically contribute to such functions. RANKL is expressed in mesenchym al cells such as bone marrow stromal cells (BMSCs) and osteoblasts.

The Osteoclast Differentiation Factor This was identified even before the origin and formal naming of the branch, the fieldof "osteoimmunology." More thanthree decades ago, antigenstimulated immune cells were shown to produce soluble factors such as inte rleukin (IL)1 that stimulate osteoclastic bone resorption. Since the 1980s, it has been observed tha t osteoblast lineage cells and bone marrow stromal cells of mesenchymal lineage are involved in the regulation of osteoclast differentiation in the bone marrow microenvironment, indicating tha t osteoclastogenesissupporting mesenchymal cells provide certain factors essential for osteoclast differentiation. Burger et al. found that osteoclasts could be developed using an in vitro cocultur e of embryonic bone rudiments and hematopoietic cells. Since embryonic bone rudiments contai n chondrocytes as well as osteoblasts and osteocytes, the result suggested that these cells are invo lved in the regulation of osteoclastogenesis. Another in vitro coculture system for osteoclastoge nesis has been established and is widely used. This coculture system requires celltocell contact be tween the osteoclast precursor cells of monocyte/macrophage lineage and osteoblast lineage cells derived from calvarial bone. Therefore, it was hypothesized that osteoclastogenesis supporting cells should express an osteoclast differentiation factor (ODF) as a membrane protein.

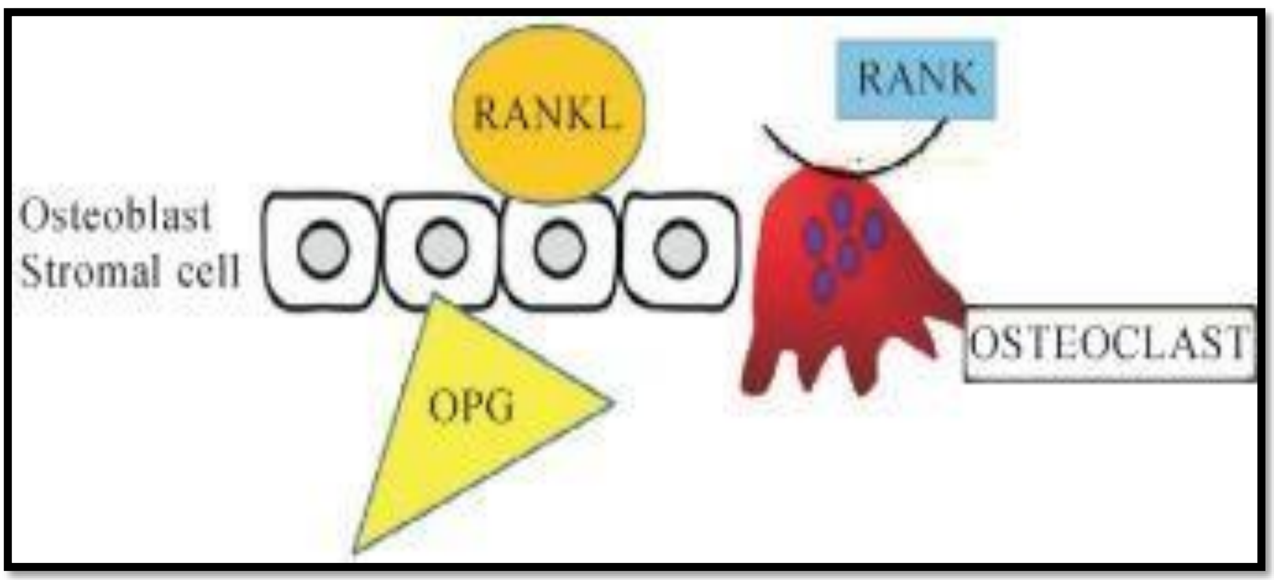

Figure 7: Cells, ligands, receptors and decoys

CHAPTER 4: OSTEOIMMUNOLOGY IN BONE AND IMMUNE DISEASES

The interconnenction: immune system and bone in physiology is maintained in pathologic al conditions and some of the diseases found associated with the osteoimmunology disease are

1. **Rheumatoid Arthritis.** Rheumatoid arthritis (RA) affects 12% of the populatio n and involves an autoimmune reaction with an autoantibody response to citrul linated proteins (and others such as rheumatoid factor and collagen type II). R A is characterized by synovitis involving angiogenesis, synovial proliferation, in creased infiltration, survival, and decreased apoptosis of inflammatory cells. Fur ther to this there is an increase in osteoclast number and activity leading to fo cal bone erosions, juxtaarticular osteopenia, and joint destruction. Animal model s suggest that there may also be suppression of localised osteoblast formation of bone.
2. **Periodontitis:** Periodontitis is a inflammatorychronic disease of the gingival tissues, with an associated loss of the supporting structures including the periodontal ligamen t and alveolar bone. The aetiology involves an inflammatory response to bacterial inf ection such as P. gingivalis and possibly an autoimmune reaction, as reviewed. Perio dontitis is the most common and widespread bone loss pathology in humans with 64 % of the US population aged 65 years and older reported as having moderate or seve re periodontitis. Despite the prevalence of this disease the most common treatment is either mechanical subgingival plaque removal or surgical debridement. Inevitably, in the absence of effective treatment, support structures (periodontium) are compromis ed and the affected teeth will loosen and fall out. Periodontitis and RA have the simila r pathophysiology, characterized by destructive inflammation that culminates in locali zed bone loss. The citrullination of proteins by P. gingivalis and the subsequent genera tion of autoantigens that drive autoimmunity in RA have been proposed as a possible mechanism linking these two diseases.
3. **Postmenopausal Osteoporosis**: Osteoporosis is a disorder characterized by compr omised bone strength and deterioration of bone quality and compromised bone stre ngth, often leading to fragility (low trauma) fractures. The impact of this

terms of cost, morbidity and mortality. According to data from the National Health and Nutrition Examination Survey (2005,2008), 9% of adults age 50 and older had osteoporosis at the femur neck or lumbar spine. About 47% had low bone mass at either site. The impact of this disorder is significant in terms of cost, morbidity and mortality. Estrogen is a hormone important for not only the development and maintenance of the female reproductive system, but also bone homeostasis. In postmenopausal women, a reduction in the estrogen level results in rapid bone loss and an increased risk of bone fractures. Bone cells, including osteoclasts, osteoblasts, and osteocytes, express the nuclear estrogen receptor. Besides, estrogen exerts multiple effects on immune cells including lymphocytes, macrophages, and dendritic cells, which also express nuclear estrogen receptors, it is necessary to consider the complex interactions that exist among estrogen deficiency, bone loss, and the immune system.

4. **Bone Fracture Healing:** Bone fracture healing is physiological process in which the ibone consistently returns to its original shape, structure, and mechanical strength. In response to tissue injury, a multistage repair process is initiated for the regeneration of bone. Bone regeneration comprises a cascade of events involving blood clot formation, inflammation, callus generation, primary bone formation, and secondary bone remodeling. Immediately after injury, the first stage of bone healing, hematoma formation, starts with vessels disruption,plateletaggregation,andbloodcoagulationintheinjurysite.Thehematoma contains immune cells such as neutrophils, macrophages, and lymphocytes. Removal of the hematoma after injury results in significantly delayed healing, suggesting a key role of the immune cells and their cytokines in fracture repair. An inflammatory response is initiated coincidentwiththehematomaformation.

5. **Myelodysplasia and Acute Myeloid Leukemia:** Another strong link between bone and the immune system is the fact that osteoblasts can influence the progression of preneoplastic and neoplastic transformations in the myeloid lineage. In fact, osteoblasts are able to slow down leukemia progression in mouse, creating an unfavorable microenvironment for leukemic blast growth. Consistently, osteoblast number is reduced by more than half in leukemic patients. Simulating this situation by mouse genetics, causes leukemic blasts to grow faster and engraft better. The same authors demonstrated that osteoblasts have another tight link to human leukemia: osteoblasts that have been genetically engineered to express a constitutively active form of β catenin, are able to induce leukemic transformation in myeloid cells, causing MyeloDysplaSia (MDS) and then Acute Myeloid Leukemia (AML). The concept of bone cells inducing malignant transformation, however, was not new, since already a few years earlier, Raaijmakers and colleagues found that ablating the miRNA processing protein dicer from osteoblast progenitors induces dysfunctional haematopoiesis, eventually leading to MDS and AML development. The field of “niche-induced leukemia” has received much attention, and still many groups are working on this topic to date.

CHAPTER 5: THE LATEST DEVELOPMENTS IN OSTEOIMMUNOLOGY

Despite much of the field has already emerged, several groups are still actively discovering new mole cules that can be considered part of the osteoimmunology world. This has been the case for a secret ed protein named homologous to Lymphotoxin, exhibits Inducible expression and competes with H SV Glycoprotein D for binding to Herpesvirus entry mediator, a receptor expressed on T lymphocyt es (LIGHT, a.k.a. Tumor Necrosis Factor SuperFamily member 14, TNFSF14), which has been link ed to increased bone resorption in osteoarthritis more than 10 years ago, and has known a renaissan ce in the last few years as target for bone loss and biomarker for bone disease in multiple myeloma. This molecule seems to have a dual effect in bone: high levels are linked to bone loss, and so is its absence. The mechanisms involving it are therefore quite complex This behavior is also common to another regulator of bone mass that has recently emerged in the last few years: LipoCaliN-2 (Lcn2). This protein is also called Neutrophil Gelatinase-Associated Lipocalin (NGAL), as it can bind MMP9, a crucial factor for neutrophil extravasation. Furthermore, Lcn2 is also r eadily overexpressed during inflammation, and following treatment with TNFα, IL17, and I L1β, and its role in inflammatory diseases is only starting to emerge; what is sure is that t his molecule can be considered a player in innate immunity. In 2009,Lcn2 is strongly over expressed in osteoblasts following in vitro mechanical unloadingwhich led to the concept that Lcn2 is a mechanoresponsive gene regulating bone homeostasis. Surprisingly, removi ng this protein reduced bone mass. This is mostly due to the fact that Lcn2 impairs imp airs energy metabolism when removed,osteoblasts when overexpressed, which causes an i ndirect osteoblast dysfunction. The role of Lcn2 in bone is still under investigation byoth er groups where it has been found to influence hematopoiesis, and the melatonin receptor MC4R.

CHAPTER 6: SUMMARY AND PERSPECTIVES

The bone is a organ that responds to a variety of exogenous stimuli and regulates itself according to various environmental cues such as tumors,calcium intake, aging, mechanostressand infections. Th e bone carries diverse functions related to the endocrine,skeletal,and immune systems, and research is carried out in various fields but, to understand multifunctional organs like bone, it is necessary to unite the findings and k nowledge obtained fromeach discipline. Osteoimmunology is a good example of such interdisciplina ry unification. In this sense it is no wonder that osteoimmunology is covering an increasingly wider fi eld, because once one sees the crucial connections, it is obvious why it is crucially important to inve stigate anything related to either bone or immunology from the unified viewpoint of osteoimmunol ogy. In the future, it will be intriguing to analyze mechanicallyand elucidate the evolutionally relati onships between bone and the immune system. The concept of osteoimmunology is not only of crucial importance for such issues in basic biology, but also the development of novel therapeutic strategies in joint and bone diseases as well as immune disorders.

CHAPTER 7: CONCLUSION

The concept of osteoimmunology is aging well, almost 20 years since the term was coined. This wa y of interpreting bone and the immune system has been steadily providing new insights about how t he two of them operate and cooperate. As an example, the role of pro-inflammatory cytokines in promoting osteoclastogenesis, and the many parallelisms between immu ne cells and osteoclasts have proved crucial to understand the biology of these giant bone-eating cells. Intriguingly, the control mechanisms between bone and the immune system are tightly interconnected,complex; complexity of this field has made it difficult for researchers to find result, the kind that leads to the direct clinical application. Nevertheless, thanks to the effort of many s cientists, nowadays clinics can use drugs, classically employed to treat osteoporosis, for im munological diseases. In conclusion, although the study of osteoimmunology has provided many answers, it also raised more questions, which we need to answer in order to underst and the field in a vast way.

CHAPTER 8: REFERENCES

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