Chapter 1
Osteoimmunology: The Bone-Immune Crosstalk

1.1 Historical Background

The term osteoimmunology was first used in 2000 by Professors Arron and Choi (2001). This monograph does not seek to repeat our knowledge of the fundamental inter-relationship between the bone and the immune systems. Rather, the intent of this writing is to expand our understanding of the implications and applications of this novel aspect of the basic sciences by integrating it in the emerging priorities of evidence-based clinical decision-making and comparative effectiveness research.[1]

Case in point, the recent report by the Agency for Healthcare Research and Quality (AHRQ; ahrq.gov) on the process of inflammation of joints, such as the knee joint in primary and secondary osteoarthritis, and the elucidation of the best available evidence for treatment effectiveness, with emphasis on risk-benefit and benefit-cost assessments (*vide infra*).

The purpose of this monograph is to explore the fundamentals of the bone-immune crosstalk as they pertain, for example, to the case outlined above of an inflammatory process proximal to, and detrimental to bones and joints. This monograph then discusses in greater depth the process by which the best available evidence

[1] AHRQ defines comparative effectiveness research as "…designed to inform health-care decisions by providing evidence on the effectiveness, benefits, and harms of different treatment options…."; by contrast, AHRQ makes the statement that, "within the context of clinical decisions, (clinical prediction rules) pull in aspects of the history and physical exam and in an evidence based fashion estimate… or make treatment recommendations…". One can distinguish, therefore, comparative effectiveness research and analysis from clinical decision-making that is evidence-based, that is based on the best available evidence, on the grounds that the former's bottom line relates to cost-effectiveness, cost-benefit ratio, and risk-benefit ratio, and is primarily a utility-based process aimed at increasing the likelihood of success of treatment for the lowest cost and risk for a patient group with a given set of symptoms and diagnostic criteria. By contrast, evidence-based decision-making, while certainly incorporating concerns of costs, is primarily directed by the clinical needs and wants of the patients and the clinician's expertise, and involves an inductive/deductive process of logic in designing the optimal treatment of any one given patient, in an unquestionably patient-centered intent, format, and delivery (Chiappelli and Cajulis 2009; Chiappelli et al. 2009).

F. Chiappelli, *Osteoimmunopathology: Evidence-Based Perspectives from Molecular Biology to Systems Biology*, DOI 10.1007/978-1-4419-9495-0_1,

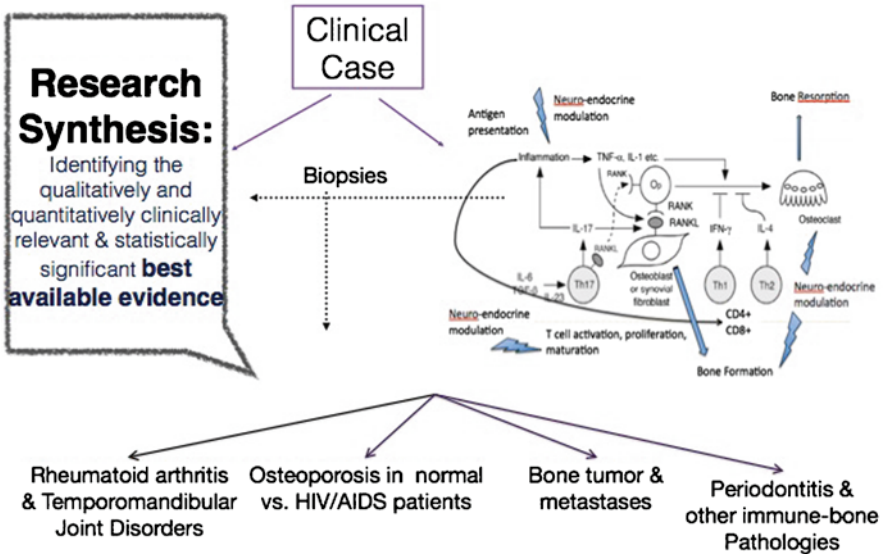

Fig. 1.1 Translational evidence-based osteoimmunology. The figure presents the fundamental paradigm of this book. The model is one that initiates with a clinical case, which, in the biological context of this work, relates to the inter-connections between the bone system and the immune system. The essential cellular and molecular components of the bone (=osteo) – immune interaction, "osteoimmunology" – are presented in the insert to the figure, and developed in the pages of this book. The figure outlines the process by which, starting from a clinical case that relates to osteoimmunology, the best available evidence for treatment efficacy and for benefit effectiveness can be obtained by means of the research synthesis design. The translational nature of this approach, with emphasis to applications and implications to a variety of osteoimmunopathologies, which range from osteoarthritic, to ospetoporosis, bone tumor immune surveillance, and other bone immunopathologies (e.g., osteonecrosis of the jaw) are noted, as they pertain to the content of the chapters that follow

is sought, analyzed, obtained, and utilized, as exemplified by the report cited above. In brief, this monograph conjoins the cutting-edge biology of osteoimmune interactions with the cutting edge science of research synthesis in an effort toward advancing the timely and critical emerging domain of translational evidence-based osteoimmunology (Fig. 1.1).

Osteoimmunology, as a scientific discipline for research and clinical practice, is merely one decade old at the time of this writing. In that span of time, a fast-growing collection of articles on specific domains of fundamental research in osteoimmunology has been published, which documents and describes the fundamental mechanisms of interactive communication between the bone and the immune systems, as well as clinical implications and applications. The generation of this cumulative knowledge was culminated by the publication of a book chapter by Professors Weitzmann and Pacifici of Emory University at Atlanta, GA (Weitzmann and Pacifici 2005a, Chap. 6), as well as a comprehensive review of the field, first in 2005 (Weitzmann and Pacifici 2005b; Lorenzo and Choi 2005), and the seminal

paper by Professor Cohen (2006). In 2007, we recall as an elegant comprehensive review article coauthored by Drs. Rauner, Sipos, and Pietschmann of the Ludwig Boltzmann Institute of Aging Research, the Institute of Pathophysiology at the Center of Physiology and Pathophysiology, and the Division of Veterinary Medicine of the University of Austria (Rauner et al. 2007), as well as an important update of the state of research in the field produced by Professor Jean-Pierre David (2007). Work continued to multiply worldwide in this domain of science, which led to the reviews by Drs. Julian Quinn and Hasnawati Saleh from Victoria Australia (cf., Lorenzo et al. 2008; Quinn and Saleh 2009), Professor Takayanagi at the University of Tokyo, Japan (Takayanagi 2009), Drs. Gallois, Mazzorana, Vacher, and Jurdic from the University of Lyon, France (Gallois et al. 2009), and Dr. Xing at the University of Rochester, NY (Xing 2009), to cite only a few salient excellent overviews of the field in the recent past (cf., Nakashima and Takayanagi 2009).

The first International Conference on Osteoimmunology: Interactions of the Immune and Skeletal Systems was held in Crete, Greece (May 28-June 2, 2006), and the proceedings, which provide an important overview and introduction to the basics of the field were published by Springer-Verlag (Choi 2007). The second International Conference on Osteoimmunology was held in Rhodes, Greece (June 8–13, 2008). Professor Yongwon Choi of the University of Pennsylvania again edited the proceedings of the conference (Choi 2009). The third International Conference on Osteoimmunology: Interactions of Immune and Skeletal Systems just met, at the time of this writing, at the Nomikos Conference Center in Fira, Santorini, Greece (June 20–25, 2010). We should look forward to the proceedings of that meeting shortly.

1.2 Physiology

1.2.1 The Bone Talks to the Immune System

The most prominent functions of bone are the protection of internal organs and the support of body structures. Beyond those functions, bone additionally serves as an attachment site for muscles allowing locomotion and as an appropriate cavity for hematopoiesis in bone marrow. As a reservoir for inorganic ions (e.g., calcium), bone is responsible for the maintenance of calcium homeostasis and is able to rapidly mobilize mineral stores on metabolic demand. Bone is a connective tissue composed of cells and extracellular matrix, the latter being further subdivided into an inorganic, and an organic component, which is mineralized. Its main constituent is type I collagen (~95%), and in minor amounts other types of collagens, noncollagenous proteins, and proteoglycans. The inorganic matrix of bone predominantly contains calcium and phosphorus in the form of hydroxyapatite crystals ($[3Ca_3(PO_4)_2](OH)_2$) deposited and amassed into the collagenous matrix. The interdigitation of the two matrices confers the characteristic rigidity and strength to the bony skeleton,

while preserving some degree of elasticity. Rather than an inert and static material, bone, the major constituent of the skeleton in all vertebrates, is a highly organized living tissue whose metabolism is intimately intertwined with, cross-regulates, and is modulated by several of the major physiological systems.

The immune system, and the cells and factors that constitute it, provides an essential and integral part of the underlying metabolism of bone. These processes are reviewed in the following sections. In brief:

- *Osteo-nervous interactions* pertain to the observation that bone is richly innervated by both autonomic and sensory neurons, serve sensory and regulatory functions, and mediate bone cell and immune cell activities directly (Warden et al. 2005).
- *Osteo-endocrine interactions* refer to our understanding of the extent to which hormones (e.g., adrenocorticotropin hormone [ACTH]; Isales et al. 2010; parathyroid hormone [PTH] and calcitonin; Carter and Schipani 2006) regulate bone mass by directly influencing the metabolism of bone-regenerating (i.e., osteoblasts) and bone-resorbing cell populations (i.e., osteoclasts) (*vide infra*), modulating either the collagen mass (i.e., ACTH) or the calcification content (i.e., PTH, calcitonin). PTH raises blood calcium levels by stimulating bone resorption. Calcitonin reduces blood calcium by suppressing bone resorption and increasing osteoid calcification. The osteoid is the matrix secreted by osteoblasts and osteocytes prior to mineralization. Calcitonin acts by directly inhibiting osteoclast activity via the calcitonin receptor. Calcitonin receptors have been identified on the surface of osteoclasts. Calcitonin directly induces inhibition of osteoclastic bone resorption by affecting actin cytoskeleton, which is needed for the osteoclastic activity. In brief, the peptide hormone PTH is one of the most important regulators of calcium ion homeostasis (Kronenberg et al. 1993; Potts et al. 1997). In response to low blood calcium levels, PTH is secreted into the circulation and acts on kidney, bone, and intestine to maintain blood calcium concentrations. In bone, PTH upregulates the production of the pro-inflammatory cytokine, interleukin (IL)-6 and of the Receptor Activator for Nuclear Factor κ B Ligand (RANKL; also known as TRANCE: Tumor Necrosis Factor [TNF]-related activation-induced cytokine, OPGL: osteoprotegerin ligand, and ODF: osteoclast differentiation factor) (*vide infra*) by osteoblasts, thereby facilitating the differentiation, activation, and survival of osteoclasts (Huang et al. 1998; Dai et al. 2006). Consequently, PTH, as well as PTH-related protein (PTH-rP), promotes bone resorption and de facto the release of calcium (Pollock et al. 1996; Onyia et al. 1997). The active hormonal form of vitamin D (aka, calcitriol, 1,25-dihydroxycholecalciferol, 1,25-dihydroxyvitamin D_3, VitD), is essential for the development and maintenance of the mineralized skeleton (Dardenne et al. 2001; Panda et al. 2004). Osteoblast number, bone formation, and bone volume increase serum alkaline phosphatase levels, and are associated with a decreased production of RANKL, but an enhanced production of the osteoclastic cytokine osteoprotegerin (OPG; also known as OCIF: osteoclastogenesis inhibitory factor) (*vide infra*) (Kitazawa et al. 2003). Regarding bone homeostasis,

estrogen and androgens are the most intensively investigated sex steroids and, in contrast to PTH and Vit D, enhance bone formation and inhibit bone resorption (Hofbauer et al. 1999; Khosla et al. 2002; Leder et al. 2003). Estrogen as well as testosterone deficiency inevitably lead to an increased rate of bone turnover (Jilka et al. 1998), as well as simultaneous increases in osteoclastic precursors and early osteoblastic precursors. Estrogen deficiency results in a net loss of bone as a consequence of an increased production of RANKL and a decreased production of OPG by osteoblastic cells, as well as the enhancement of the secretion of pro-inflammatory and pro-resorptive cytokines by lymphocytes such as IL-1, IL-6, and TNF-α (Pacifici et al. 1991; Jilka et al. 1995; Eghbali-Fatourechi et al. 2003). The bone-protective effect of estrogen is mediated in large part by transforming growth factor (TGF)-β, which induces apoptosis of osteoclasts (Oursler et al. 1991; Hughes et al. 1996; Fox and Lovibond 2005). From the viewpoint of research synthesis for evidence-based and comparative effectiveness analysis, as discussed in greater details in Chap. 3, it is important to note at this juncture that in at least two randomized controlled trials from the Women's Health Initiative, hormone replacement therapy (HRT) was shown to decrease the incidence of major osteoporotic fractures. Serious undesirable side effects such as cardiovascular disease and cancer have occurred and therefore other medications are used nowadays in the treatment of osteoporosis. Raloxifene is a selective estrogen receptor modulator (SERM) and is approved for the treatment of osteoporosis. Like estrogen, SERMs are known to mediate their effects through the estrogen receptor. While estrogen binds equally strongly to α- and β-receptors, raloxifene preferentially binds to the α-receptor (Women's Health Initiative 2002, 2004). From the perspective of translational clinically relevant complex systematic reviews (Chiappelli et al. 2010a, b), this specificity to a certain estrogen receptor will have implications in the evidence-based revision of clinical practice guidelines because it points to a fundamental biological process that allows a higher affinity to bone, and therefore the risk of side effects of SERMs are less pronounced than those of HRT (Riggs and Hartmann 2003). Similarly, as bone metabolism is inter-dependent with the endocrine system, and it is regulated and modulated by the central and peripheral nervous systems (cf., neuroendocrinology), it is not surprising to find that neuropeptides (e.g., neuropeptide Y, NpY; gastrointestinal peptides, etc.) modulate osteoblasts and osteoclasts, and have direct regulatory effects upon bone metabolic activity (Lee and Herzog 2009; Sousa et al. 2009; Wong et al. 2010).

- *Osteoimmune interactions* refer to the involvement of immune cells and the factors they produce (e.g., cytokines, *vide infra*) as they modulate bone metabolism, as is discussed in greater depth in the remainder of this book. Suffice to state at this juncture that since osteoclasts are derived from the monocyte/macrophage (=myeloid) lineage, the macrophage-osteoclast interaction is of prime importance in bone metabolic regulation. Certainly, evidence is mounting in support of osteoclast interaction with T cells, B cells, and dendritic cells (Takayanagi 2010). Moreover, the emergence and replenishment of the immune system throughout the life span occurs as hematopoietic precursors develop in the bone marrow, a

process that is finely regulated within the neural-immune-hematopoietic axis by neuropeptides of the tachykinins family (e.g., substance P, neurokinin [NK]-A) (Greco et al. 2004; Murthy et al. 2007). Evidently, these physiological facets interplay with the two natural kinds of mature bone: cortical compact bone and medullary spongy bone.

- *Compact and dense cortical bone* typically composes most of the thickness of long bone shaft, the diaphysis (Gr: dia, through; π, phusis, structure, formation) is made of compact bone. Micro-anatomical studies reveal that compact cortical bone is composed of cylindrical units of bone structure, the osteons with a dimension of circa 0.2 mm in diameter. Osteons together form the Harvesian system, named after the British physician and anatomist Clopton Havers (1657–1702). Each osteon consists of concentric lamellae of bone matrix, mainly collagen fibers, surrounding the central Harvesian canal. These micro-anatomical structures form canal-like structures, which Havers himself termed Canals of Havers in his *Osteologia Nova*, (Havers, 1691). Blood vessels and nerves course the canals, and thusly penetrate the bone tissue. The long axis of osteon is usually parallel to the long axis of the bone, but the collagen fibers in the different lamellae of the osteon are oriented at different angles and provide increased strength and elasticity of the osteon units. Volkmann's canals, named after the German physiologist and anatomist Alfred Wilhelm Volkmann (1800–1877), constitute an alternative series of channels that provide a route for blood vessels and nerves to reach the principal osteonal canal, and that link together into a network that inter-connects the Harvesian canals across different osteons. This micro-anatomical network ensures the physiological homogeneity of bone metabolism, and the continuity of circulatory and nervous supply to the bone marrow cavity. Within that histological contextual milieu, osteoblasts develop and mature into osteocytes, each living within its own micro-environmental *lacuna*. Osteocytes make contact with the cytoplasmic processes of their counterparts across *lacunae* via a network of even smaller canals, the *canaliculi*, which together form a structure that facilitates the exchange of nutrients and metabolic waste among osteocytes and osteon units. In brief, nutrients and other substances, including hormones, neuropeptides and cytokines, pass from blood vessels into the Harvesian canal, through an increasingly finer *reseau* of channels, eventually to distant osteocytes. Inner and outer circumferential *lamellae* run the length of the shaft located at the inner and outer surface of the long bone. Osteocytes sit in the *lacunae* between the *lamellae*, and as such can be involved in both bone deposition (i.e., osteoblasts and mineralization) and bone resorption (i.e., osteoclasts and bone degradation), both of which are modulated (i.e., increased under certain conditions, and decreased in other cases) by products associated with the cell-mediated immune system (e.g., cytokines, cytokine receptors, membrane-bound and soluble clusters of differentiations [CD's; cf., Notes], and the like). Interstitial lamellae form as remnants of previous osteons, and reflect the process of constant bone modeling, resorption, and remodeling (i.e., bone metabolism), which is orchestrated by the interaction of bone cells with cells of immune system within and about these micro-anatomical structures of osteons, canals, *caniculli*, *lamellae,* and *lacunae*.

- The *spongy, cancellous, trabecular, medullary bone* is composed of similar cells and matrix structure, and in that resembles compact bone. However, micro-anatomical examination reveals a distinct lamellar structure of collagen in spongy bone, which is typically not arranged concentrically around a central canal, but rather as *lamellale* that run parallel to one another. Consequently, spongy bone is composed of bone spicules, the *trabeculae*, which are endowed of varying shapes and sizes. The space between the spicules is filled with the bone marrow, the flexible tissue found in the hollow interior of bones. That space is rich in nerve endings, blood vessels, and capillaries. This micro-anatomical histological "geographical" distribution is critical in the formation of niches, where cells of the bone system – the osteoprogenitor cells at the external and internal surface of the bone – and cells of the immune system – the hematopoietic progenitor cells – develop, interact, communicate, and cross-feed in a fundamental osteoimmune concert of epigenetic events (*vide infra*).
- The *bone marrow* is the underlying contextual framework wherein the interaction between the bone and the immune system commences. The red marrow consists mainly of hematopoietic tissue, whereas the yellow marrow, which constitutes the stroma of the bone marrow, contains principally adipocytes.
- *Compact and spongy bone interact*: Small congregates of spongy bone result that are facing the marrow cavity, the large medullary cavity. The two expanded ends of the long bones, the anepiphyses (Gr: ana, end-piece; phusis, structure, formation), consist mostly of spongy bone covered with a thin shell of compact bone. The calvarium and the sternum are two flat bones made of two layers of relatively thick compact bone, and an intervening layer of spongy bone.
- Bone is lined externally by a dense connective tissue, the *periosteum*, composed histologically of an outer fibrous layer, rich in fibroblasts, and an inner osteogenic layer, rich in periosteal and endosteal cells that give rise to the osteoblast population. The inner side of bones is lined by the endosteum. There is no periosteum lining at the joints of long bones. In remodeling bone, periosteal cells work in concert with bone modeling factors, including cytokines and other immune factors, to increase bone width and length. In nonremodeling bone by contrast, that surface is lined by a layer of flat bone lining cells, which appear quiescent and incapable of osteogenesis. It is believed that these cells still may function as nutritional support for osteocytes that are embedded in the underlying bone matrix.
- *Endosteal cells* line the marrow cavities in compact bone, and the spicules of spongy bone. In a manner akin to the periosteal cells, endosteal cells act as osteoprogenitor cells, and are modulated by immune factors to give rise to osteoblasts. Indeed, research has established that two steps occur in bone remodeling, which involve the endosteal cell layer: resorption of a volume of bone by osteoclasts is followed sequentially by the formation of a comparable volume by osteoblasts. However, the regulatory processes that initiate, sustain, and terminate this sequence are intertwined, and intimately regulated by signals, which travel along the osteocyte canalicular system to endosteal lining cells, and

they entertain a complex molecular cross-talk that involves precursors, mature cells, cells of the immune system, and products of both the resorbed matrix and cellular immune products that titrate each other, and modulate each other in a concerted and finely orchestrated cross-system, multicellular remodeling machinery toward the end-result of either removing or forming a net volume of bone, osteoprecursors, or hematopoietic precursors (Martin and Seeman 2008; Matsuo and Irie 2008). Bidirectional signaling and interaction is likely to occur and to continue among osteoblasts, osteoclasts, and endosteal lining cells throughout the lifespan, during which time bone metabolism is both sustained and modulated by immune factors, and results in osteoblastic bone formation with mineralization of bone matrix, and osteoclastic bone resorption with apoptosis and demineralization.

- The *bone remodeling cycle* involves a complex series of sequential steps that are highly regulated. The "activation" phase of remodeling is dependent on the effects of local and systemic factors on mesenchymal cells of the osteoblastic lineage. These cells interact with hematopoietic precursors to form osteoclasts in the "resorption" phase. In the later "reversal" phase, mononuclear cells appear on the bone surface, complete the resorption process, and produce the signals that initiate formation. In successive waves, mesenchymal cells newly derived from the adult stem cell pool differentiate into functional osteoblasts, which lay down matrix in the next "formation" phase. Case in point, new research demonstrates that vascular endothelial cells can transform into multipotent stem-like cells, which by all account resemble mesenchymal stem cells, by means of an activin-like kinase-2 (ALK2) receptor–dependent mechanism: activation of the ALK2 pathway (cf., Notes) in endothelial cells by means of ligands such as with the ligands transforming growth factor-β2 (TGF-β2) or bone morphogenetic protein-4 (BMP4), led to a distinct endothelial-to-mesenchymal transition and acquisition of a stem cell-like phenotype (Medici et al. 2010) (Fig. 1.2).
- *Osteoblasts* are immature bone cells, specifically mononucleate bone-forming cells that descend from osteoprogenitor cells. Osteoblasts derive from a common family branch that arises from mesenchyme, a type of loose connective tissue, derived from the three embryonic germ layers (i.e., endoderm, mesoderm, ectoderm). The mesoderm is the proper germ layer that gives rise to the skeleton and the hematopoietic system. The mesenchymal prominent ground substance matrix contains a loose aggregate of reticular fibrils and unspecialized cells, which are capable of developing into bone, cartilage, lymphoid organs, and the circulatory system (Strum et al. 2007). Core binding factor 1 (Cbfa1) the runt-related transcription factor 2 (runx2), and the downstream factor osterix (Osx) are critical transcription factors for lineage commitment of stem cells toward osteoblast differentiation (Ducy et al. 1997; Caetano-Lopes et al. 2007). Although a relatively rare event, it is not excluded that, given the appropriate microenvironment, osteocytes and osteoblasts can revert back to earlier stages of their development. Note that most current research has established that endothelial cell-derived mesenchymal stem cells may be an efficient

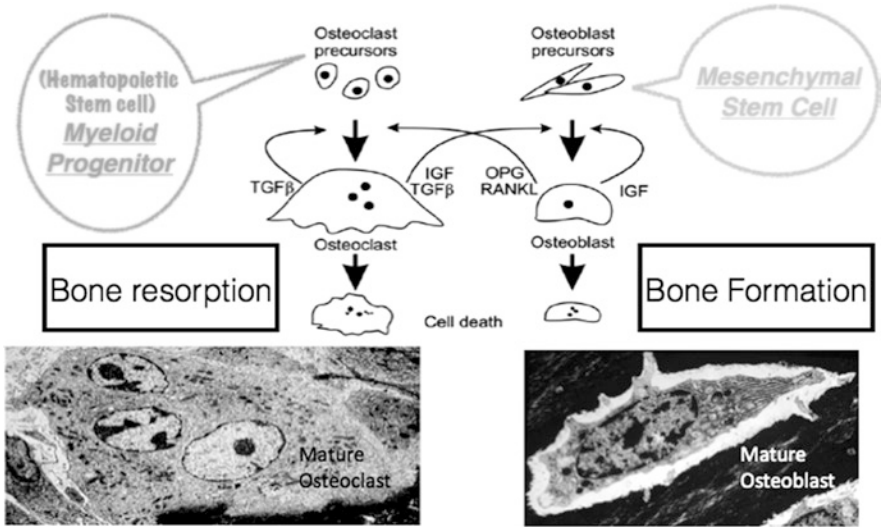

Fig. 1.2 Bone metabolism. The figure shows a composite of electron micrographs of, respectively, a mature human osteoclast, and a mature human osteoblast. Characteristically, the relative myeloid-related complexity of the cytoplasmic compartment of the former contrasts with the relative smoothness of the mesenchyme-derived osteoblast cytoplasm. Integrated in the figure is a diagrammatic representation of the maturation process of both populations from their respective myeloid osteoclat precursor and mesenchyme osteoblast precursor. The central aspect of the figure represents the fundamental core of bone metabolism. The balance between bone formation by osteoblasts and bone resorption by osteoclasts is regulated by immunological factors, such as tumor growth factor (TGF)β, insulin-like growth factor (IGF), osteoprotegerun (OPG), which blocks receptor activator of NF-kB ligand (RANKL) signaling in osteoclasts. It is important to note that among the families of regulatory proteins for bone metabolism, a large body of research has emerged in the most recent decade on the role of the family of Wnt's, which are secreted cysteine-rich glycoproteins that locally activate receptor-mediated signaling pathways, in part because mutations of Wnt's that lead to either gain or loss of function of the Wnt coreceptor lipoprotein receptor-related protein 5 (Lrp5) induce significant changes in bone mass (e.g., high bone mass) or osteoporosis, respectively. It is now clear, moreover that the "canonical" (i.e., b-catenin-dependent; b-catenin is an intracellular anchoring protein involved in cell adhesion through E-cadherin) Wnt signaling pathway regulates the lineage progression for the maturation of osteoblasts, and represses osteoclastogenesis by increasing osteoprotegrin (OPG) expression, thus altering the OPG/RANKL ratio. Adapted from: ebi.ac.uk/biomodels-main/static-pagesdo?page=ModelMonth%252FOctober2007%252FBIOMD0000000148_MM; endotext.org/parathyroid/parathyroid1/parathyroid1.html

source of cells for regeneration of bone and cartilage (Horwitz 2010). Indeed, preliminary results indicate that endothelial cell–derived mesenchymal stem cells may actually be superior as a cell source for bone and cartilage cell therapy for patients requiring regenerative medicine interventions (Medici et al. 2010).

- Osteoblasts are located on the surface of *osteoid* seams and make a protein mixture known as the osteoid, which mineralizes to become bone. The osteoid seam is a narrow region of newly formed organic matrix, not yet mineralized,

located on the surface of a bone. Osteoid is primarily composed of Type I collagen produced by the osteoblasts, which also manufacture hormones and prostaglandins that mediate bone metabolism for the formation of the immediately surrounding matrix.

- The principal *proteomic signature of osteoblasts* is the production and expression of alkaline phosphatase (EC 3.1.3.1), a key dephosphorylating enzyme that contributes to the accumulation of calcium and phosphate into the vesicles generated during the formation of the matrix. Four principal isozymes of alkaline phosphatase have been recognized. The alkaline phosphatase, tissue-nonspecific isozyme is found in bone, and is encoded by the ALPL gene, located on chromosome 1. Alkaline phosphatase is a membrane-bound glycosylated enzyme that is not expressed in any particular tissue and is, therefore, referred to as the tissue-nonspecific form of the enzyme. When missing, the disorder known as hypophosphatasia arises, which manifests as hypercalcemia and skeletal defects (Swallow et al. 1998).
- Osteoblasts also produce bone sialoprotein, a 60–80-kDa small integrin-binding ligand, N-linked glycoproteins (SIBLING), protein constituent of mineralized tissues such as bone, dentin, cementum and calcified cartilage, osteocalcin (also known as BGLAP: bone γ-carboxyglutamic acid-containing protein), and osteopontin (OPN; also known as also known as BSP-1 or BNSP: bone sialoprotein I, ETA-1: early T-lymphocyte activation, or SPP1: secreted phosphoprotein 1). OPN is a remarkable member of the SIBLING family that is particularly interesting to osteoimmunologists because, while produced by osteoblasts and critical of osteoblastic function, it is also expressed by a variety of immune cells, including macrophages, neutrophils, dendritic cells, and T and B cells. In the immune system, OPN is reported to be endowed with chemotactic properties that promote cell recruitment to inflammatory sites, adhesion properties by means of binding to several integrin receptors, a function that favors cell attachment and contributes to promoting cellular immune activation of T cells and cytokine production, as well as cell survival and regulation of apoptosis. The SIBLING protein family is a group of noncollagenous proteins whose members (e.g., OPN, BSP) appear at distinct phases of development, which suggest substantial differences in the distribution of the SIBLING proteins between organic and inorganic phases, and divergent molecular and epigenetic regulatory roles in osteogenesis and immune regulation of osteogenesis (Weber and Cantor 1996; Huang et al. 2008; Wang and Denhardt 2008; Sun et al. 2010) (cf., notes).
- In brief, therefore, and by their very nature, osteoblasts occupy a central place in the *osteoimmune dialog*: they support hematopoiesis, and when completely surrounded by matrix, osteoblasts become osteocytes, which can both secrete and resorb matrix, and thus are responsible for maintaining the bone matrix itself. In that respect, they act as mechano-sensory receptors of the osteoimmune entity, capable of regulating the bone's response to stress and mechanical load. Osteocytes are typically poor in organelles, indicating other primary functions than matrix synthesis and mineralization. Further maturation of osteocytes alters their morphology into cells projecting dendritic processes. These channel-like structures enable osteocytes to communicate with each other, may, among other

functions, act as mechanosensors to permit bone to react to environmental challenges. Loaded vesicles eventually rupture, and increase local concentration of minerals that contributes to initiate the mineralization process. Osteoblasts and osteocytes synthesize the collagen-rich organic matrix that provides the optimal conditions for matrix mineralization by secreting numerous bone matrix proteins and matrix metalloproteinases (MMP). They eventually engage in programmed cell death, apoptosis (cf., notes), which leads to increased secretion of osteoclastogenic cytokines that favor bone resorption (Gu et al. 2005; Kogianni et al. 2006). It is now clear that apoptosis is not the only regulated cell death program involved in the concerted modulation of tissue homeostasis and the removal of unwanted cells in biological organisms. Other cell death modalities, and their cross-talk, require increased understanding (Zhivotovsky and Orrenius 2010), particularly as they pertain to immune-mediated bone degenerative diseases.

- By contrast, *osteoclasts* are responsible for remodeling of bone to reduce its volume – that is, of bone resorption. These large multinucleated cells – tissue-specific giant polykaryons – arise from monocyte common progenitors, and can actually arise from monocyte/macrophages directly, and are thus not related to the same family lineage of bone forming cells as osteoblasts. Osteoclasts are multinucleated, giant cells of hematopoietic origin formed by the fusion of mononuclear preosteoclasts derived from myeloid cells. Fusion-mediated giant cell formation is critical for osteoclast maturation: without it, bone resorption is inefficient. The d2 isoform of vacuolar (H+) ATPase (v-ATPase) V0 domain (Atp6V0d2) is one of the principal regulators of osteoclast fusion and bone formation. Similar to the myeloid family, osteoclasts are endowed with phagocytic properties, share several families of plasma membrane receptors with certain immune cell populations, and function in a manner similar to their mature myeloid equivalent. They are found on bone surfaces in what are called Howship's *lacunae*, named after John Howship (British surgeon, 1781–1841). These structures are bone resorption pits that result following the breakdown of the bone surface and consequential erosion of the bone by the osteoclasic enzymes, including lysosomes, organic acids, and hydrolytic enzymes. The osteoclastic layer that contacts the bone is divided into the microvillus structure, a ruffled border rich in plasma membrane folding, and a ring-like perimeter of cytoplasm, termed the clear zone, which marks the area of bone in the process of being resorbed.
- The *metabolic function of osteoclastic structures*, which we recall from our introductory statements, is modulated by the neuroendocrine system, such that an increase in PTH levels, and a decrease in calcitonin is associated with an increased number and functionality of osteoclasts. PTH is secreted by the parathyroid glands as an 84 amino acid polypeptide, which acts to increase the serum concentration of calcium (Ca^{2+}). Calcitonin is a 32-amino acid linear polypeptide hormone produced primarily by the parafollicular cells, the C-cells of the thyroid, which are located adjacent to the thyroid follicles and reside in the connective tissue. They appear large and with a characteristic pale stain, compared with the follicular cells or colloid. Calcitonin acts to counter PTH, and to reduce blood calcium.

- *Osteoclastic factors* shared with the hematopoietic cell lineage briefly noted in our introductory remarks include:
  - *OPG* (i.e., osteoprotegerin), the OCIF is a cytokine-like 401 amino acid peptide found either as a 60-kDa monomer or 120-kDa dimer linked by disulfide bonds, first reported as a protein that exposed an osteopetrotic phenotype when overexpressed in transgenic mice, which was secreted by preosteoblasts/stromal cells, which could inhibit osteoclast development and activation (hence the name attributed to its bone-protective effects: osteoprotegerin) (Simonet et al. 1997). OPG belongs to the TNF receptor superfamily, but lacks the transmembrane and cytoplasmic domain. OPG is a cytokine expressed in a variety of tissues (e.g., lung, heart, kidney, liver, stomach, intestine, brain, spinal cord, thyroid gland, smooth muscle tissue, and, in addition to osteoclasts, certain immune cells – in fact, the expression of OPG by dendritic cells, a population of antigen-presenting cells, was shown to increase with immune maturation, to be stringently dependent upon NF-κB signaling, and to act in concert with regulatory processes of immune responses in lymphoid tissues (Schoppet et al. 2007)). Nonetheless, the principal osteoimmune role of OPG has been assigned to bone protection by impairing the function and maturation of osteoclasts. This is obtained by OPG binding to RANKL on osteoblast/stromal cells, thus blocking the RANKL–RANK ligand interaction between osteoblast/stromal cells and osteoclast precursors, thereby blunting the differentiation of the osteoclast precursors into mature osteoclasts (Khosla 2001; Boyce and Xing 2007). OPG also promotes cell survival, in certain physiological situations, by inhibiting TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis (Reid and Holen 2009).
  - The *production of OPG is subject to neuroendocrine regulation*. Specifically, production of OPG is stimulated in vivo by the female sex steroid estrogen, and accounts for the biochemical mechanisms by which estrogen is the predominant sex hormone that slows bone loss. As the production of estrogen becomes irregular, and decreases with age (i.e., menopause), the likelihood of osteoclast-mediated bone resorption, and consequentially osteoporosis (*vide infra*), increases. One treatment modality for osteoporosis includes the drug strontium ranelate, which both increases deposition of new bone osteoblasts and reduces the resorption of bone by osteoclasts. In fact, fundamental research has established that strontium ranelate acts by means of activation of the calcium- or other cation-sensing receptor, by increasing the expression of OPG, coupled with decreasing RANKL expression by the osteoblasts (Hamdy 2009).
  - *RANKL* is the ligand for the receptor activator for Nuclear Factor |B, RANKL is also known as TRANCE: TNF-related activation-induced cytokine, OPGL: ligand for OPG, and ODF. The expression of OPG and RANKL is modulated by various endocrine, immune, inflammatory, and neuroendocrine-immune modulators, including PTH, estrogens, glucocorticoids IL-6, IL-8, IL-11, INF-γ, TGF-β, prostaglandin (PG)-E2, bone morphogenetic protein (BMP)-2, VitD, and many others (Khosla 2001). The extensive distribution of RANKL throughout the body attests for its multiple functions, among which the

most important – at least in our context presently – is the induction and regulation of osteoclastogenesis. RANKL is a surface-bound molecule on certain immune cells, and on osteoblasts that serves to activate osteoclasts. Overproduction of RANKL has been implicated in a variety of degenerative bone diseases (e.g., rheumatoid arthritis, psoriatic arthritis). Targeted silencing of the related gene in a murine experimental system leads to a lack of osteoclasts and associated osteopetrosis. RANKL, which is also expressed by memory T helper cells, plays a role in dendritic cell survival and maturation, and modulates the cellular immune system by contributing to the regulation of T cell activation, proliferation, and maturation. Activated T cells induce expression of the RANKL gene, which, in the osteoimmune context, contributes to osteoclastogenesis and increase bone resorption and loss. The human RANKL gene has been localized to chromosome 13q14 and encodes for three isoforms: RANKL1 and RANKL2 are type II transmembrane proteins, and RANKL2 also possesses a shorter intracellular domain. RANKL3 is a soluble protein, partially produced by the cleavage of the membrane-bound form by TACE (TNF (converting enzyme, a metalloprotease; cf. notes)).

– The *RANKL proteomic signature* pathway includes the activation of RANK following binding of its ligand (RANKL), and leading to activation of AKT/PKB. This is an enzyme complex endowed with antiapoptotic serine/threonine kinase, protein kinase B (PKB) activity, which is also called AKT.[2] As of now, three genes have been identified in the Akt family: Akt1, 2, and 3, which code for enzymes that are members of the serine/threonine-specific protein kinase family (EC 2.7.11.1). Akt1 is involved in cellular survival and it inhibits the apoptotic processes. Akt1 also induces protein synthesis pathways, and, in general, tissue growth and development. Akt2 is an important signaling molecule in the insulin signaling pathway, and is required to induce glucose transport. Akt3 is predominantly expressed in brain, and mediates the regulation of neurogenesis. The Akt enzyme family possesses a protein Pleckstrin Homology (PH) domain, which allows it to bind to phosphoinositides with high affinity, thus effecting the kinase signaling pathway. In this manner, once correctly positioned in the membrane via binding of PIP3, Akt is phosphorylated by its activating kinases (i.e., phosphoinositide dependent kinase 1 (PDPK1) at threonine 308, and mammalian target of rapamycin complex 2 (mTORC2), a phosphatidylinositol 3-kinase-related kinase, at serine 473). The mTOR phosphorylation events stimulate the subsequent phosphorylation of Akt by PDK1. Phosphatidylinositol-3 kinase (PI3K)-dependent Akt activation can be regulated through the tumor suppressor phosphatase and tensin homolog (PTEN). Akt may also be activated in a PI 3-kinase-independent, and cAMP-dependent protein kinase A (PKA)-dependent activity. That is to say, convergent

[2] The Akt nomenclature is rather unusual, if not enigmatic as it is not descriptive of a function per se: "Ak" was a temporary classification name of a murine strain with spontaneous thymic lymphomas, and "t" was simply meant to refer to "transforming" (Staal et al. 1977).

pathways ensure activation of AKT/PKB, because, once activated, this enzyme complex can go on to activate or to deactivate its myriad substrates via its kinase activity, and to regulate cellular survival and metabolism by binding and regulating a plethora of downstream effectors. The AKT/PKB pathway engaged by RANKL is critical to the regulation of several cellular processes in bone and in immune cells through a multipronged proteomic profile.

– *RANK*, the Receptor Activator of Nuclear Factor κB, is a member of the TNF receptor family, which also includes CD120 (TNFα receptor), CD40, Fas, and CD34. The family of proteins, which consists to date of 6 members (TRAF1-6), is endowed with complex functional properties in that they converge in regulating inflammation, antiviral responses, and apoptosis by mediating transmembrane signaling not only for the members of the TNF receptor family, but also for the members of the Toll/IL-1 family. TRAF proteins interact with several protein kinases including IRAK1/IRAK, SRC, and PKCz, and thus establish a critical regulatory link between distinct signaling pathways. Thus, and in the specific context of the present discussion, RANK signaling, initiated by RANKL, can be transferred to a parallel cascade via TRAF-6, which, upon activation engages signaling pathways leading to the activation of the transcription factors NFAT, NFkB, the MAP kinase mediators jun, fos, and p38 as well as the down-stream targets of Akt AFX/FOXO4. The two most investigated pathways are the activation of the transcription factors NF-κB and AP-1 (activated protein 1). Targeted disruptions of the p50/p52 component of NF-κB and the c-fos component of AP-1 result in impaired osteoclastogenesis and an osteopetrotic phenotype (Kobayashi et al. 2001; Ye et al. 2002). Together, these signals (cf., notes) contribute to osteoclast differentiation, activation, and survival. Indeed, RANK is a type I membrane protein expressed by osteoclasts, as well as by dendritic cells, and involved in bone resorption and the facilitation of immune signaling. RANK-deficient mice show similar phenotypes to those of RANKL knock-out mice, including osteopetrosis and missing lymph nodes (Dougall et al. 1999).

Furthermore, we must note in the osteoimmune spectrum:

- *M-CSF*: the macrophage-colony-stimulating factor is principally a cytokine that regulates hemopoietic stem cell differentiation into macrophages and related cell types, hence, its role osteoclast differentiation. M-CSF binds to the Colony stimulating factor 1 receptor, (CSF1R; aka, macrophage colony-stimulating factor receptor, M-CFSR, CD115), a tyrosine kinase transmembrane receptor of the CSF1/platelet-derived growth factor (PDGF) receptor family.
- *Osteocalcin* is the BGLAP, a noncollagenous protein of bone and dentin, encoded by the BGLAP gene on chromosome 1. As a hormone, osteocalcin induces the beta cells of the pancreas to increase release of insulin, and directs adipocytes to release the hormone adiponectin, which increases sensitivity to insulin (Lee et al. 2008). Indeed, it is now evident that, through the uncarboxylated form of the osteoblast-derived factor osteocalcin, bone regulates glucose metabolism and fat mass, and thus has a central homeostatic role, not only for bone metabolism. Specifically, research has now established a putative working

model, which stipulates that osteoblastic function is negatively regulated by the adipocyte-produced hormone leptin. When bound to its receptor in the central nervous system (CNS), leptin exerts a stimulation of the sympathetic nervous system. Leptin deficiency leads to increased osteoblast activity and increased bone mass. By contrast, expression of Esp gene by osteoblasts regulates glucose homeostasis and adiposity by regulating osteocalcin, which mediates both pancreatic insulin and adipocytic adiponectin production for the overall modulate of energy metabolism (Wolf 2008).

- *COX2*: cyclo-oxygenase 2, the prostaglandin-endoperoxide synthase 2, acts both as dioxygenase and as a peroxidase. It is key in the biosynthesis of the prostanoids; that is, prostaglandins, prostacyclin, and thromboxanes, which contribute to the inflammatory and mitogenesis cascades. It presents as two isoenzymes: a constitutive form, COX-1, and its inducible counter-part, COX-2, which, in humans, is expressed in a limited number of cell types and regulated by certain specific stimulatory events. Interestingly, the product of the COX-2 peroxidase function on arachidonic acid, prostaglandin H2 (PGH2) is a central precursor to this family of pro-inflammatory molecules: PGH2 is converted by prostaglandin E2 synthase into PGE2, by prostaglandin D2 synthase into prostaglandin D2, by thromboxane-A synthase into thromboxane A2, and by prostacyclin synthase to create prostacyclin. Among other functions, and specifically to the focus of this writing, PGE2 contributes to the fever response, and stimulates osteoblasts to release factors to bone resorption by osteoclasts (Watkins et al. 2003; Li et al. 2006). By contrast, PGD2 recruits TH2 cells, contributes to the development and the exacerbation of allergic diseases, and, at least with respect to body temperature, acts to oppose PGE2. Note: PGH2 is also produced by COX-1. Aspirin irreversibly inhibits COX-1, thus preventing the formation of PGH2, and therefore thromboxane A2, thus blocking its regulation of the activation of new platelets and increased platelet aggregation. PGH2 contrasts the effects of prostacyclin (PGI2), which chiefly prevents formation of the platelet plug involved in primary hemostasis (i.e., blood clot formation).
- *FLAP*: The 5-lipoxygenase-activating protein (FLAP) activates the 5-lipoxygenase enzyme (aka, arachidonate 5-lipoxygenase, 5-lipoxygenase, 5-LO, Alox5), a member of the lipoxygenase family, which transforms essential fatty acids into leukotrienes. Again, two major groups of products, result that have counter-balancing effects upon the inflammatory process: arachidonic acid yields the 4-series cysteinyl leukotrienes (LTB4, LTC4, LTD4, LTE4) that are generally pro-inflammatory and make up the slow-reacting substance of anaphylaxis (SRS-A). Leukotrienes; eicosapentaenoic acid yields the 5-series Leukotrienes (LTB5, LTC5, LTD5, LTE5) that generally favor an antiinflammatory response. Whereas the role of leukotrienes, and therefore of FLAP, in immune regulation is evident, their significance to bone metabolism is unclear at this time. Nonetheless, emerging evidence indicates, as one would expect from an osteoimmune viewpoint, that bone formation and resorption are under the subtle control of multiple regulatory systems that include prostaglandins and leukotrienes (Pilbeam et al. 2002). Leukotrienes

are fatty molecules, naturally produced eicosanoid lipid mediators of the immune system that contribute to inflammation, and whose production generally accompanies the production of histamine for triggering and exacerbationg allergic reactions. In the presence of factors stimulating bone resorption, the production of PGE2 by COX-2 is favored in osteoblasts. Osteoclastogenesis is inhibited by the reduction of IL-1-induced COX2 activity and PGE2 production, in an essentially RANKL-independent process (Ha et al. 2006; Hiraga et al. 2006; Shoji et al. 2006). Additionally, LTB4 also favors osteoclastogenesis in a RANKL-independent manner (Traianedes et al. 1998; Anderson et al. 2001; Jiang et al. 2005).

In summary, therefore, it is becoming increasingly apparent that, as much as the emergence of immune cells via hematopoiesis occurs in bone, and through bone metabolism, products of the immune system influence bone metabolism, generation, and loss through resorption. It is also evident now that bone pathologies arise, more often than not, from immune reactions (e.g., inflammation), or engender immunopathologic responses, which in turn can exacerbate bone disease. The question that has become intertwined in this discussion at this juncture is, therefore, the manner in which the immune system might "talk" to bone metabolism.

1.2.2 The Immune System Talks to the Bone

Osteoimmunology, we now must all agree, represents a conceptual rethinking of multiple phenomena, interrelating biological events in bone and the immune system. The root of exploration of this interplay begins with the basic understanding that the bone environment is critical for the development of hematopoietic stem cells, from which the cells of the immune system derive, and that various immunoregulatory cytokines influence the fate of bone cells.

There is general agreement that lymphocytes influence bone remodeling by exerting an impact on osteoclastogenesis. Thus, T cells are assumed to be responsible for bone loss which occurs as a consequence of a series of pathological conditions, for example systemic viral infections and chronic local bone and joint diseases, such as rheumatoid arthritis or inflammatory bowel disease. Concerning the type of impact that T cells exert on osteoclastogenesis results from in vitro and in vivo experiments differ to a high degree. The same is true for different lymphocyte subpopulations, i.e., data concerning the effect of CD4 and CD8 lymphocytes on osteoclastogenesis, are not consistent.

On the one hand, data from literature suggest an inhibitory effect of T cells. In one in vitro study, Vit D-stimulated osteoclast-like cell formation was enhanced after lymphocyte depletion. This was attributed to increased PGE2 production and consecutive upregulated RANKL and downregulated OPG expression. IFN-γ was found to be the modulatory factor, which is produced by activated anti-CD3 T cells, and which interferes with TRAF6, thus strongly inhibiting the RANKL-induced activation of NF-κB and JNK in vitro. It was acknowledged that resting T cells

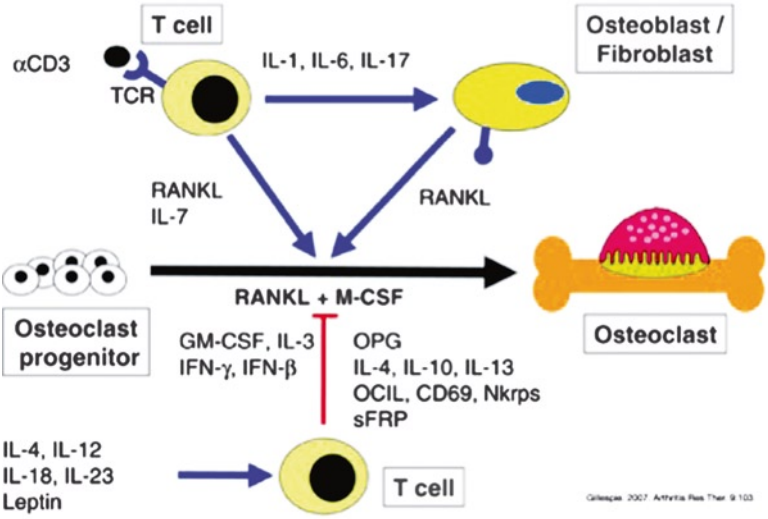

Fig. 1.3 Cytokines regulating T cells, osteoblasts, and osteoclasts. This schematic representation, adapted from Gillespie, 2007, shows to some degree the intimacy between the cellular immune system and bone metabolism. The central role played by RANKL in osteoimmune interaction is evinced

typically exert no effect on osteoclastogenesis (Grcević et al. 2000). T cells indeed have no effect in coculture with IFN-γR−/− BMMs (bone marrow-derived monocyte/macrophage precursor cells) stimulated by RANKL (Takayanagi et al. 2000).

By contrast to the above-mentioned results, there are cases where resting T cells may also negatively regulate osteoclastogenesis via production of granulocyte/monocyte colony-stimulating factor (GM-CSF) and IFN-γ by CD4 but not CD8 T cells (Shinoda et al. 2003). Another in vitro study demonstrated that the downregulatory effect of lymphocytes is due to the CD8 T cell subset, and effect at all independent of IL-4 and TGF-β (John et al. 1996) (Fig. 1.3).

Nonetheless, activated T cells promote and induce osteoclastogenesis both in vitro and in vivo. CD4 T cells stimulated by conjugated anti-CD3 with anti-CD28 costimulus exert their effect via membrane-bound and secretory RANKL, and inhibited osteoclastogenesis, whereas T cells activated with staphylococcal enterotoxin A, PHA, and Con A had inconsistent effects. The osteoclastogenic effect was CD4+ Tcell –dependent (Wyzga et al. 2004).

So the problem remains as to what degree can all the lines of evidence be taken as concerted and confluent, rather than contrasting and contradictory. Much fundamental research remains to fully characterize the cellular immune regulation of osteoclastogenesis.

- *Osteoblasts/stromal cells* are the main regulators of osteoclastogenesis. They express the cytokines RANKL, which binds to RANK on osteoclast precursors and thereby induces osteoclastogenesis, and OPG, which is able to prevent that

interaction. Among others, TGF-β and 17-β-estradiol stimulate the production of OPG whereas Vit D, PTH, and PGE2 promote the production of RANKL. Upon activation via dendritic cells T cells activate osteoclasts directly through the secretion of sRANKL. Furthermore, T cells secrete INF-γ, which on the one hand stimulates macrophages to produce pro-inflammatory cytokines, which in turn promote RANKL expression in osteoblasts/stromal cells, and on the other hand suppresses permanent osteoclast activation by the destruction of TRAF6. But, these might be circumstantial and secondary to allied event, since, for example, we also know that endothelial cells have been shown to express RANKL and OPG and might therefore also participate, or perhaps even initiate and direct in the regulation of osteoclastogenesis (Yasuda et al. 1998).

- In the murine system, *T cells* do not seem to be absolutely required for osteoclastogenesis in a rheumatoid arthritis animal model, although they contribute to form an important pathologic feature in arthritic joints (Plows et al. 1999). These observations suggest either that the experimental animal model is not true in mirroring the human pathology, or that there exist fundamental species differences, perhaps deriving from the nonabsolute congruency between murine and human cellular immune processes, mechanisms, and cell populations and subpopulations. That said, it is unquestionable that cells in rheumatic joints are in a distressed state, attributable in part at least to IFN-γ. It follows therefore that pro-inflammatory cytokines act detrimentally upon synovial fibroblasts, across species and pathological model. These cells in the activated state – activated T cells, challenged synovial fibroblasts – are the main source of RANKL, which in turn is responsible for osteoclast differentiation, although RANKL has also been shown to be produced by T cells.
- *Cytokines* have a myriad of effects upon osteoclastogenesis: IL-1α, IL-1β, IL-6 and other members of the gp130 cytokine family, IL-7 (*vide infra*) and TNF-α directly or indirectly promote osteoclastogenesis, whereas IFN-β, IFN-γ, IL-3, IL-4, IL-10, IL-13, and IL-12 alone and in synergy with IL-18 inhibit osteoclast formation. The IFN-γ-mediated suppression of osteoclastogenesis, most likely occurs, as noted above, by inhibiting RANKL signaling by downregulation of the transcription factor TRAF6 expression via the signal transducers and activators of transcription family member 1 (Stat1). TGF-β, depending on the micro-environment, can both induce, via suppressor of cytokine signaling 3 (SOCS3) or suppress osteoclastogenesis (Theoleyre et al. 2004; Takayanagi et al. 2005). Osteoblasts may also serve in these activation processes since they posess antigen-presenting properties, express both MHC Class-II molecules and CD54 (ICAM-1) and CD166 (ALCAM), and are thus capable also of activating T cells. Osteoblasts express members of the TOLL-like receptor family (i.e., TLR-4, TLR-5, and TLR-9), indicating an active role in host immune response. Pattern recognition receptors were found not only on the surface of osteoblasts but also intracellularly (Marriott et al. 2005). The data could demonstrate the expression of the nucleotide-binding oligomerization domain proteins NOD1 and NOD2 following bacterial challenge of the cells. Osteoblasts also produce IL-6 upon encountering T cells and following stimulation by IL-17 (*vide infra*). Lastly, and of great relevance to osteopathologies, such as rheumatoid

arthritis, which often have an etiology that can be traced to a superantigen origin, osteoblasts are quite capable and endowed to present superantigen effectively to T cells. (Stanley et al. 2006). Nonetheless, there is relatively little detailed information on the cytokine production pattern of osteoblasts. IL-6 was shown to be produced by stromal cells/osteoblasts (Bordin et al. 2003). Production of IL-6 and RANKL by osteoblasts is promoted by PTH and TNF-α, but with markedly different kinetics. Whereas PTH induces a rapid, but transient elevation of both cytokines, TNF-α leads to a biphasic and sustained increase of these cytokines, thus indicating the potent role of TNF-α in osteoimmune pathologic conditions (Dai et al. 2006).

In brief, bone homeostasis refers to the constant process of remodeling, by which old bone is replaced by new bone. The skeleton, including the rostral skeleton is a metabolically active organ that undergoes continuous remodeling throughout development and aging. Two populations of cells drive this process: the bone-resorpting osteoclasts, and the bone-generating osteoblasts. Several factors control and regulate the process of bone homeostasis, including:

- Local factors, such as immune cell-produced cytokines (e.g., TGF-β; TNF-α; IL-1β; IL-12).
- Growth factors (e.g., M-CSF; BMP's).
- Mediators of cell-to-cell and matrix-to-cell communication.
- Products of the neuroendocrine system (e.g., estrogen, PTH; PTHrP; insulin-like growth factors [IGF's]) (Raisz 1999; Hadjidakis and Androulakis 2006).

As was discussed earlier and now can begin to fit into a *Gestalt*[3] of our understanding of the fiundamentals of oesteo-immunology. In brief, the RANK/RANKL/OPG system is critical to the processes of bone resorption and formation, are tightly coupled to allow the wave of bone formation to follow each cycle of bone resorption, and are the central engine that regulates bone and skeletal integrity (Hadjidakis and Androulakis 2006).

1. The regulation of bone metabolism occurs principally through the RANK signaling pathway. Stromal osteoblastic precursors express on their surface the RANK ligand (RANKL) as we have discussed earlier, and produce a soluble of RANKL, osteoprotegerin (OPG). Bone-resorbing hormones, including PTH, and cytokines, such as IL-1β or TNF-α, induce RANKL expression by stromal osteoblastic cells. RANKL then engages RANK on the osteoclastic progenitors, which induces their differentiation into osteoclasts. The process is enhanced by M-CSF, which is also produced by the osteoblast cells, and finds receptor-mediated binding and cell signaling on the maturing osteoclastic cells. OPG is a decoy ligand, preventing RANKL interaction with RANK on osteoclastic progenitors, and acts as a potent inhibitor of ostaclastogenesis.
2. The osteoimmune relationship notably manifests expression of RANKL by activated T cells, and T cells support osteoclastogenesis. OPG secretion is upregulated

[3] German for "shape," or totality of a given entity in terms of its shape. Used commonly to describe wholeness.

by anti-CD3 antibody stimulation of normal CD4 T cells in vitro, and enhanced by IL-4, IL-1β, TNF-α, GM-CSF, but blunted by IL-10 (Kotake et al. 2001; Colucci et al. 2004.

3. Other immune factors can substitute RANK/RANKL/OPG system in osteoclastogenesis. Bacterial lipopolysaccharide (LPS), the CD34 ligand, leads to inflammation systemically via IL-1β, IL-6, and TNF-α, and can stimulate osteoclastogenesis and bone resorption locally by binding to the Toll-like receptor 4, leading to NF-κB activation in OC. This effect is not blocked by OPG. Further, in combination with TNF-α, the inflammatory T-cell-derived cytokine found in fluid from osteoarthritic joints, IL-17 can stimulate OC differentiation and bone degradation in an NF-κB-independent process that also cannot be blocked by OPG.
4. The observation that T cells from arthritic joints also express RANKL suggests that IL-17 may be a significant pathway of osteoclastogenesis that is redundant with, but independent from the RANK/RANKL/OPG system (Fig. 1.4 and Table 1.1).

    - The *IL-17 family of cytokines* includes IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F. The primary function of IL-17-related cytokines is to modulate induction of many immune signaling molecules. The most notable

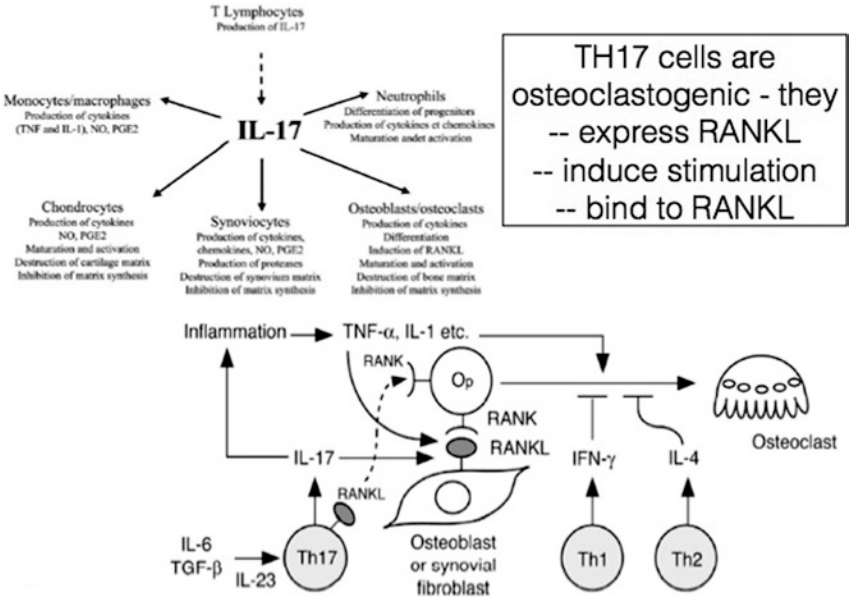

Fig. 1.4 The role of interleukin (IL)-17 in the osteoimmune system. This schematic representation of the multifaceted physiologic role of IL-17 shows that this inflammatory cytokine, produced by activated lymphoid-derived T cells rather than myeloid-derived monocytes/macrophages, contributes to the activation of the latter, as it also contributes to regulating the maturation of osteoclast precursors (Op) and, when further aided by T cell-produced TH1 and TH2 cytokines, the activation of bone resorbing osteoclasts. Much of the regulatory role of IL-17 is attributed to its modulation of RANKL. Adapted from: osteoimmunology.com/

Table 1.1 Modulators of RANKL, OPG, and RANK expression (taken from Weitzmanm and Pacifici 2007)

	RANKL	OPG	RANK
Hormones			
Vitamin D3[a]	↑	↑	↑
PTH	↑	↓	
PTHrP	↑		
Estradiol		↑	
Testosterone		↑	
Prolactin	↑	↓	
Cytokines			
TNFα	↑	↑	
TNFβ		↑	
IL-1α		↑	
IL-1β		↑	
IL-6[b]	↑	↑	↓
IL-11	↑	↑	
IL-17	↑		
CD40L	↑	↑	
Growth factors			
TGF-β	↓	↑	↓
BMP-2	↑	↑	
LIF	↑	↑	–
IGF-I	↑	↓	
VEGF			↑

role of IL-17 is its involvement in inducing and mediating proinflammatory responses. IL-17 is commonly associated with allergic responses. IL-17 induces the production of many other cytokines (such as IL-6, G-CSF, GM-CSF, IL-1β, TGF-β, TNF-α), chemokines (including IL-8, growth related gene alpha, GRO-α, and MCP-1), and prostaglandins (e.g., PGE2) from many cell types (fibroblasts, endothelial cells, epithelial cells, keratinocytes, and macrophages). The release of cytokines causes many functions, such as airway remodeling, a characteristic of IL-17 responses. The increased expression of chemokines attracts other cells including neutrophils but not eosinophils. IL-17 function is also essential to a subset of CD4+ T-Cells called T helper 17 (TH17) cells. As a result of these roles, the IL-17 family has been linked to many immune/autoimmune related diseases including rheumatoid arthritis, asthma, lupus, allograft rejection, and antitumor immunity. Each member of the IL-17 family has a distinct pattern of cellular expression. The expression of IL-17A and IL-17F appear to be restricted to a small group of activated T cells, and upregulated during inflammation. IL-17B is expressed in several peripheral tissues and immune tissues. IL-17C is also highly upregulated in inflammatory conditions, although in resting conditions is low in abundance. IL-17D is highly expressed in the nervous system and in skeletal muscle and

IL-17E is found at low levels in various peripheral tissues (Aggarwal and Gurney 2002; Kolls and Linden 2004; Yu and Gaffen 2008). Although it has only limited homology to other cytokines, IL-17 exhibits proinflammatory properties similar to those of TNFα, particularly with respect to induction of other inflammatory effectors, including several bone pathologies, most notably rheumatoid arthritis (Gaffen 2004). Research has established that signal transduction pathways dependent on PI3K/Akt and NF-κB are involved in bone-related pathology mediated IL-17 (Kim et al. 2005), possibly by modulating, in part at least, production of related pro-inflammatory cytokines (i.e., IL-6, IL-8) by synovial fibroblasts (Hwang et al. 2004).

- The *IL-17 receptor family* consists of five, broadly distributed receptors that present with individual ligand specificities. Within this family of receptors, IL-17R is the best described. IL-17R binds both IL-17A and IL-17F and is expressed in multiple tissues: vascular endothelial cells, peripheral T cells, B cell lineages, fibroblast, lung, myelomonocytic cells, and marrow stromal cells. IL-17RB, binds both IL-17B and IL-17E. Furthermore, it is expressed in the kidney, pancreas, liver, brain, and intestine. IL-17RC is expressed by the prostate, cartilage, kidney, liver, heart, and muscle tissues. The IL-17RC gene may undergo alternate splicing to produce a soluble receptor in addition to its cell membrane-bound form. In similar manner, the gene for IL-17RD may undergo alternative splicing to yield a soluble receptor. This feature may allow these receptors to inhibit the stimulatory effects of their yet-undefined ligands. The least-described of these receptors, IL-17RE, is known to be expressed in the pancreas, brain, prostate, and bone (Aggarwal and Gurney 2002; Gaffen 2004; Kolls and Linden 2004; Yu and Gaffen 2008).
- Lymphocytes expressing γδ T-cell receptors constitute an entire system of functionally specialized subsets that have been implicated in the regulation of immune responses, including responses to pathogens and allergens, and in tissue repair. γδ T cells represent a small subpopulation of T cells that, unlike aβ T cells, function more as cells of the innate immune system. γδ T cells are known to mediate the production of inflammatory cytokines, including interferon-γ, tumor necrosis factor-α, and interleukin (IL)-17, and thus enable the activation of other subsets of infiltrating effector cells. However, not much attention was paid to γδ T cells until the recent discovery of a distinct CD4+ T helper (TH) cell, TH17 cell. CD4+ T cells, upon activation and expansion, develop into different TH-cell subsets with different cytokine profiles and distinct effector functions. T cells were earlier divided into TH1 or TH2 cells, depending on the cytokines they produce. A third subset of IL-17-producing effector TH cells, called TH17 cells, has been discovered and characterized recently. Since then the literature on IL-17-producing cells has grown steadily, and several studies have focused on γδ T cells. Cytokine-mediated modulation of CNS inflammatory diseases by γδ T cells in humans or in animal models is currently the subject of many studies. IL-17 and its receptor IL-17R have been implicated in the pathogenesis of immune-mediated CNS diseases, and attention has been paid to understand the

mechanisms by which IL-17 cytokines and it receptor (IL-17R) family mediate the effects at a molecular level. This article reviews the studies that cover earlier aspects of γδ T cell/IL-17 biology and the new dimension of γδ T cells, IL-17, and IL-17/IL-17R signaling axis in CNS inflammation. Understanding the role of γδ T cells, IL-17, and IL-17/IL-17R signaling axis in infection and immunity could open a new avenue for immunomodulation (Das Sarma 2010; Barkhordarian et al. 2011).

- Immune cells that are endowed with the ability to respond to challenges by means of IL-17 are referred to as belonging to the TH17 group. TH17 cells are a subset of CD4+ T cells that are responsible for inflammatory and autoimmune disorders. Data demonstrate the presence of TH17 cells, some of which produce both IL-17 and IFNγ, indicating a putatively overlapping subpopulation of TH17/TH1 cells (Annunziato et al. 2007; Romagnani et al. 2009). In brief, TH17 cells are characterized by:
  - Surface expression of CCR6, IL-23R, IL-12Rb2, and CD161.
  - Expression of T-bet, retinoic acid-related orphan receptor (ROR)γτ.
  - Ability to produce IFN-γ and IL-17A in the presence of IL-12.
  - Ability to arise from CD161+CD4+ precursors, which constitutively express RORγτ and IL-23R, in response to the combined activity of IL-1β and IL-23.
  - Unresponsiveness to TGF-β for mediation of differentiation, although it can favor their proliferation by inhibiting T-bet expression (Romagnani et al. 2009).

1.2.3 The Osteoblastic Niche for Immunogenesis

The RANKL–RANK–OPG (osteoprotegerin) axis noted above is an example of an important signaling system functioning both in bone and immune cell communication. RANKL is expressed on osteoblasts and activated T cells, whereas RANK is expressed on osteoclasts, and dendritic cells, both of which can be derived from myeloid progenitor cells. Surface RANKL on osteoblasts as well as secreted RANKL provide necessary signals for osteoclast precursors to differentiate into osteoclasts. RANKL expression on activated T cells leads to dendritic cell activation through binding to RANK expressed on dendritic cells. OPG, produced by dendritic cells, is a soluble decoy receptor for RANKL that competitively inhibits RANKL binding to RANK.

The bone marrow cavity is important for the proper development of the immune system, and houses important stem cells for the maintenance of the immune system. Within this space, as well as outside of it, cytokines produced by immune cells also have important effects on regulating bone homeostasis. Some important cytokines that are produced by the immune system, including RANKL, M-CSF, TNFα, ILs, and IFNs, affect the differentiation and activity of osteoclasts and bone resorption. During chronic inflammation, the balance of bone modeling and remodeling

can be greatly affected, contributing to painful and/or visible disorders in bone metabolism.

It is also critical to recognize at this juncture that the osteoblasts provide key factors for the development of hematopoietic stem cell niches. However, there is growing evidence that bone continues to play a role in adaptive immunity at later stages beyond lymphocyte development. For example, it is now known that long-lived memory T and B cells return to specialized niches in the bone marrow. The significance of this observation is currently unknown but could be important in the crosstalk between the bone and immune system.

Hematopoietic stem cells are located in the bone marrow and are responsible for the continuous production of blood cells in an adult organism. Their capacity for self-renewal and their ability to differentiate into multiple cell types is strongly dependent on their surrounding microenvironment, which is also referred to as stem cell niche. There, cells produce various signaling molecules, cell adhesion molecules, and components of the extracellular matrix and thereby determine the long-term repopulating ability of stem cells. Taichman and Emerson (1998) remarked that osteoblasts play a crucial role in stem cell maintenance due to an intimate cell-to-cell contact via integrins (Taichman et al. 2000). Another interesting observation was made in CBFA1 deficient mice, which are devoid of osteoblasts, and characterized by the absence of bone marrow. These mice do show normal hematopoietic development in ec topic sites, such as liver and spleen until day E17.5, suggesting an important role for osteoblasts in HSC homing into the bone marrow cavity (Ducy et al. 1997; Komori et al. 1997; Otto et al. 1997).

Bone homeostasis is in turn regulated by immune responses, particularly when the immune system has been activated by infection or becomes disregulated. In conditions such as periodontitis, infiltrating lymphocytes and other mononuclear cells produce key factors, which influence bone turnover by altering the balance between bone formation, mediated by osteoblasts, and bone resorption, mediated by osteoclasts. Beyond such pathological conditions, the question of whether the immune system influences normal oral bone metabolism, either by direct or indirect mechanisms, remains unanswered. However, the discovery of RANKL, and its characterization as a key differentiation factor for osteoclasts, and the findings that RANKL is expressed on activated T and B cells, has provided critical evidence for a potential link between normal immune responses and bone turnover. It is therefore becoming clear that crosstalk between the immune system and rostral bone through activated lymphocytes and bone cells occurs throughout life, as all mammals are constantly challenged by a diverse oral microflora, which induces some level of constant low grade immune system activation. Furthermore, as the aging process unfolds, there is an accumulation of memory T cells and B cells in the bone marrow, which express RANKL on their surface. These cells have now been shown to modulate bone turnover, in particular in periodontitis and other bone degenerating diseases.

Taken together, these and related issues place oral osteoimmunology in a position of unique clinical relevance.

1.3 Implication for Stomatology

1.3.1 Facial Osteology

The rostral or facial skeleton is complex and unique in terms of the nature and structure of the bones involved, their ossification during ontogenesis, and their articulation. It corresponds to the bones of the anterior and lower human skull, as opposed to the posterior skull, the neurocranium, which contains the brain and the brain stem. The facial skeleton contains the organs proper of the anterior aspect of the cranium (e.g., eyes, ears, nose, mouth, including tongue, tonsils, etc.), and is thus often referred to as the splanchnocranium or viscerocranium.

Eight bones form the neurocranium, which are juxtaposed by means of sutures that form immovable (i.e., synarthrodial) joints. Some degree of malleability and flexibility of the sutures is permitted by the Sharpey's fibers, named after the Scottish anatomist William Sharpey (1802–1880). These fibers are not unique to neurocranial sutures, and in fact present, for example, in the attachment of the periodontal ligaments; there are micro-anatomical histological structures that resemble matrices of connective tissue intertwined together to form bundles of strong collagenous fibers, which connect the periosteum to bone.

Fourteen bones form the splanchnocranium. Moreover, encased within the temporal bones are the six auditory ossicles of the middle ear. In addition, the hyoid bone, while supporting the larynx, does not articulate with any of the other cranial bones, and thus may, or may not be considered a component of the facial skeleton proper (Fig. 1.5).

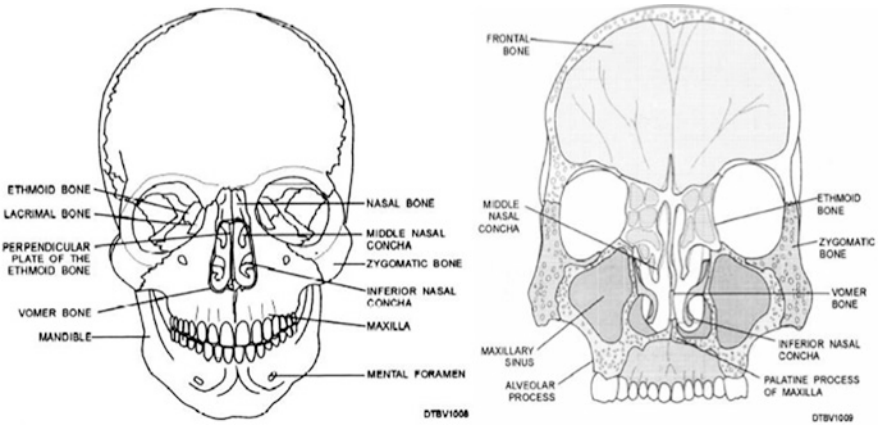

Fig. 1.5 The human facial skeleton. The figure shows a front view and a sagittal section of the facial skeleton from a human adult. Indicated are the principal bone structures that support the stoma superiorly (e.g., zygomatic, maxillary, vomer, palatine), and inferiorly (i.e., mandibular). The latter section present aspects of the deep face. Adapted from: http://www.tpub.com/content/medical/14274/

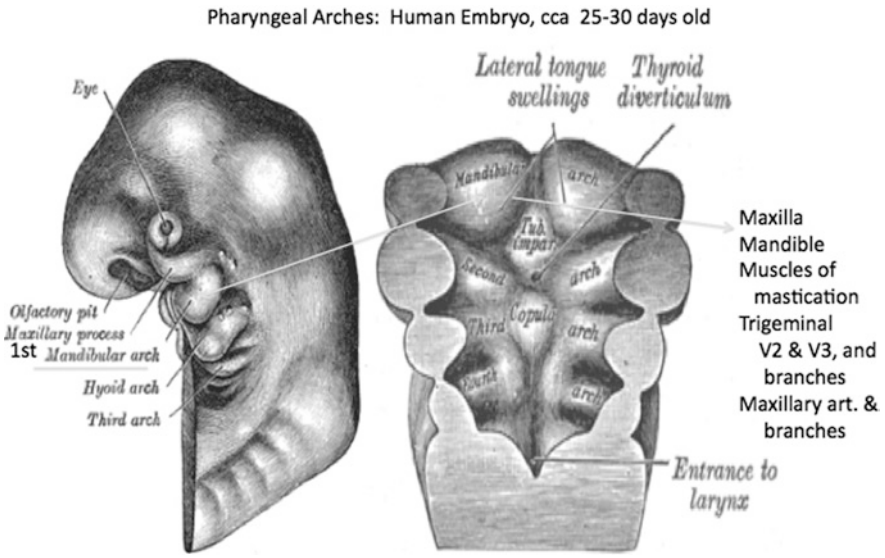

Fig. 1.6 Pharyngeal arches in the developing human fetus. The figure, adapted from Gray's anatomy, shows a side view and sagittal section of the anterio-frontal aspect of a human fetus at 25–30 days, gestational age. The specimen evinces clearly the first four tissue foldings located anterio-laterally to the median line and inferior to the developing stoma (i.e., olfactory pit). The first (mandibular) arch and associated lateral lingual tumerences are shown. The principal bones of the facial skeleton emerge from this arch. Source: Gray's Anatomy

During early ontogenesis, the bones of the facial skeleton derived from the pharyngeal arches (i.e., left and right mesodermal emergences, with no alterations in the ectodermal and endoderma layers, along the sides of the developing pharynx during the 3–5th week of intra uterine development in humans). There are six pharyngeal arches, but in human development, the fifth arch gives rise to no stomatological structures – hence, development proceeds along pharyngeal arches 1, 2, 3, 4, 6 as follows (Fig. 1.6):

- *First pharyngeal arch* – the mandibular arch: divides into a maxillary process to give rise to structures including the bones of the lower two thirds of the face (i.e., maxilla) up to and including the incus and malleus of the middle ear, as a superior regression of the Meckel's cartilage; and a mandibular process that comes to form a cartilaginous "model" of the mandible as a cartilaginous bar of the mandibular arch known as cartilage of Meckel (named after the German anatomist Johann Friedrich Meckel (1781–1833)), with the associated muscles of mastication, anterior belly of the digastric, mylohyoid, tensor tympani, tensor veli palatini, as well as the V2 and V3 branches of the trigeminal nerve and the corresponding branches of the maxillary artery. The mandible (i.e., "lower jaw") will eventually form by intramembranous ossification of the Meckel's cartilage.

- *Second pharyngeal arch* – the hyoid arch: development of the stapes ossicle of the middle ear, styloid process, hyoid (lesser horn and upper part of body), Reichert's cartilage, with the associated musculature of facial expression, buccinator, platysma, stapedius, stylohyoid, posterior belly of the digastric, as well as the facial nerve, and the stapedial artery.
- *Third pharyngeal arch* – this determines the development of the greater horn and lower part of body of the hyoid bone, as well as the stylopharyngeus muscle, the glossopharyngeal nerve, and the common and internal carotid arteries.
- *Fourth pharyngeal arch* – for the development of the thyroid and epiglottic cartilages, and the associated cricothyroid muscle, and all the intrinsic muscles of soft palate including levator veli palatini, the vagus nerve and the superior laryngeal branch, as well as the fourth aortic arches, on the right signifying the subclavian artery, and on the left signifying the aortic arch.
- *Sixth pharyngeal arch* – for the development of the cricoid, arytenoid, and corniculate cartilages, and associated intrinsic muscles of larynx except the cricothyroid muscle, the vagus nerve and its recurrent laryngeal branch, and the sixth aortic arches, on the right signifying the pulmonary artery, and on the left signifying the pulmonary artery and ductus arteriosus.

Clearly, with respect to the development of the oral cavity proper, the first pharyngeal arch, which is the first to form, separates the emerging mouth pit (i.e., stomodeum) from the pericardium, and forms pharingo-laryngeal structures. The stomodeum first arises as a depression between the developing structures that becomes the brain and the pericardium.

As the anterior aspect of the embryo forms and develops in the initial 1–4 weeks of ontogenesis, precursor rudiments of what soon develops into the cephalic flexure, the pericardial, and the bucco-pharyngeal membrane structures come to present on the antero- ventral surface of the embryo. As the brain further expands, and the forward bulging of the pericardium grows, the bucco-pharyngeal membrane forms a depression between these two prominences. This depression constitutes the stomodeum, which is lined by ectoderm, and is separated from the anterior end of the foregut by the membrane itself. The bucco-pharyngeal membrane is thus formed by the apposition of the stomodeal ectoderm and foregut endoderm. By the fourth week of intrauterine development, the bucco-pharyngeal membrane disappears, and a patent communication is established between the mouth and the emerging pharynx. The lips, teeth, and gums emerge from the ectodermal walls of the stomodeum. The tongue advances anteriorly from the floor of the pharynx: the anterior two thirds of the adult tongue derive from the first pharyngeal arch, and the posterior one third emerges from the hypobranchial eminence as the copula, formed by the forward growth and fusion of the ventral ends of the second, third, and part of the fourth arches.

Perinatally – whereas the principal features of the facial skeleton (cf., Fig. 1.5) are apparent, ontogenesis of the splanchnocranium is not finished, as stomatological ossification is not complete.

- *Inferior nasal concha*, as noted above (*vide supra*) extends laterally along the wall of the nasal cavity, and posteriorly to articulate with the conchal crest of the palatine bone. Anteriorly, it articulates with the conchal crest of the maxilla. The sphenopalatine (aka, pterygopalatine) ganglion, one of the four parasympathetic ganglion found in the deep face, lies in the pterygopalatine fossa that sits superiorly within it, and projects its branches to each of the superior, middle, and inferior ridges. The ganglion is suspended by nerve roots from the maxillary nerve, the second or middle branch of the trigeminal nerve (V2). The ganglion also has a sympathetic component, as it receives sympathetic efferent postganglionic fibers from the superior cervical ganglion. These fibers traveled from the superior cervical to the sphenopalatine ganglion via the deep petrosal nerve, which joins with the greater petrosal nerve, a branch of the facial nerve, to form the nerve of the pterygoid canal (aka, Vidian nerve, named after Vidus Vidius, the Italian anatomist born Guido Guidi, 1508–1569), that serves this ganglion. The ossification process of the nasal concha begins during the fifth month of intrauterine life from a single ossification center that emerges in the lateral wall of the cartilaginous nasal capsule.
- *Maxilla* is actually the product a fusion of two bones along the palatal fissure. On each side, the maxilla consists of the body of the maxilla, wherein lies the large maxillary sinus, the zygomatic, frontal, alveolar, and palatine processes, and the infraorbital foramen to permit passage of the infraorbital branch of the maxillary nerve (V2), and its associated artery. The maxilla articulates with two among the cranial bones (i.e., frontal and ethmoid), and several bones of the facial skeleton: nasal, zygomatic, lacrimal, inferior nasal concha, palatine, and vomer. It derives from the first pharyngeal arch, and its ossification is ossified from perhaps as many as six, or as few as two centers[4]: one for the maxilla proper and one for the premaxilla (i.e., *os incisivum*, *os intermaxillare*), which appear during the sixth week of intra uterine development. They eventually fuse completely (e.g., *sutura incisiva*), early in the third month. The suture line between the two maxillary portions may persist on the hard palate throughout adult life.
- *Palatine bone*, forms what is commonly known as the hard palate,[5] and continues posteriorly as the soft palate, which consists of a membranous aponeurosis and movable, fibromuscular fold, the velum palatini that is attached to the posterior edge of the hard palate, and extends postero-inferiorly to a curved free

[4] For an interesting account of the historical evolution of this area of research, cf., Barteczko and Jacob (2004).

[5] Note: the torus (pl., tori) palatinus (i) is a bony protrusion on the palate, most commonly found on the midline of the hard palate. Tori can also occur, abeit with a reduced prevalence, on the medial aspect of the mandibular bone (torus mandibularis). Palatal tori are more prevalent in the Asian, compared with the white Anglo-saxon US populations (20–35%). The condition is typically more prevalent in the young adult population, and tori may reduce in size in aging because of bone resorption events. The condition is twice more common in females, but relatively similar between blacks and whites in the United States. They may be an autosomal dominant trait.

margin from which hangs a conical process, the uvula. The palatine aponeurosis provides attachment for the four muscles of the soft palate: the Levator Veli Palatini, Tensor Veli Palatini, Palatoglossus, Palatopharyngeus, as well as the muscle of the uvula, the Musculus Uvulae. The palatine bone articulates with the bones of the deep face, important for the internal structure of the oral cavity, the sphenoid, ethmoid,[6] maxilla, inferior nasal concha, vomer bones. The palatine bone contributes to the floor and lateral wall of the nasal cavity, the roof of the mouth, and the floor of the orbit. Furthermore, it contributes to the formation of the pterygopalatine and pterygoid fossæ, and the inferior orbital fissure. The palatine bone is ossified in membrane from a single center, which makes its appearance about the sixth to eighth gestational week. The center appears at the angle of junction of the two parts of the bone, and spreads medially to the horizontal face, inferiorly into the pyramidal process, and superiorly to the vertical facet. These events lead to a putative timeline of ossification of the palatine bone as proceeding forward from four centers that are responsible for

- The pyramidal process and portion of the vertical part behind the pterygopalatine groove
- The remaining vertical and the horizontal portions
- The orbital process
- The sphenoidal process

At birth, the height of the vertical aspect of the palatine bone is roughly equal to its transverse width's horizontally part, and during normal postnatal ontogenic development, the former progressively grows larger than the latter.

- *Vomer bone* is a thin, somewhat quadrilateral, or trapezoidal bone situated in the median plane, and forms the inferior and posterior aspects hinder of the nasal septum. Along its two surfaces run the nasopalatine groove obliquely downward and forward to aid the nasopalatine nerve and vessels. The vomer articulates with the sphenoid and ethmoid bones, the two maxillae, and the two palatine bones, and specifically its inferior border articulates with the crest formed by the maxilla and palatine bones. The vomero-nasal organ, also called Jacobson's organ, is a chemoreceptor organ named for its closeness to the vomer and nasal bones, and is particularly developed in felines and canines. Early in ontogenesis (fifth to seventh week of gestation), the septum of the nose consists of a plate of cartilage, the ethmo-vomerine cartilage. As the postero-superior part of this cartilage is ossified, it comes to form the perpendicular plate of the ethmoid. Its antero-inferior portion persists as the septal cartilage. By contrast, the vomer is ossified in the membrane covering its postero-inferior part. Two ossific centers, one on either side of the middle line, appear about the eighth gestational week, which generates each of the two lamellae of the vomer, and which eventually joins and fuses about the third month of fetal life. A deep groove remains, which

[6] Both the ethmoid and the sphenoid bones are, as discussed above, superior and deep to the oral cavity proper, and serve more properly the nasal, rather than the oral stomata.

retains the cartilage, which is progressively absorbed in the continued process of ossification during ontogenesis as the union of the lamellae extends upward and forward. Postnatally, it takes another 10–15 years of growth and development before the lamellae is completely united to form the median plate of the vomer. The evidence of the bi-laminar origin of the vomer bone remains in adulthood in the everted alae of its upper border and the groove on its anterior margin.

- *Zygomatic bones* in vertebrates are small quadrangular paired bones which is present on each side of the face socket, forming the prominence of the cheek. They are also called in lay term, the cheekbones (aka, malar [Lt., malaris, cheeckbone] bones, malar-temporal bones). They play a critical structural role as they articulate with the maxilla, the temporal bone, the sphenoid bone, and the frontal bone. The malar aspect of the zygomatic bone presents the zygomaticofacial foramen for the passage of the zygomaticofacial nerve and vessels; the temporal aspect of the zygomatic bone supports articulation with the maxilla, and forms the anterior boundary of the temporal fossa, the lower a part of the infratemporal fossa, an irregularly shaped cavity, situated below and medial to the zygomatic arch, and bounded laterally by the ramus of mandible. During ontogenesis, the zygomatic bones ossify from three centers: the malar aspect, the superior and inferior aspects of the orbital facets, starting about seventh to eighth gestational week, and resulting in fusing by the fifth month of intrauterine life.
- *Mandible* articulates with the two temporal bones at the temporomandibular joints. These are complex synovial joints (i.e., gliding or arthrodial) whose motion, while considerably occurs in one plane only (i.e., hinge or gingymal), and which suffer from several dysfunctions, including inflammatory osteoarthritis, with significant local and systemic *sequelae*. The mandibular bone has a large and deep medullary core, where osteoimmune processes, such as those described earlier occur, as well as a cortical rim that is 2–4 mm thick is found. The ontogenic ossification of the mandibular bone is complex, and is initiated within the fibrous membrane covering the outer surfaces of the two Meckel's cartilages (right and left). The proximal (i.e., cranial) ends become contiguous with the ear capsules, as their distal extremities conjoin at the symphysis by mesodermal tissue. The process of ossification runs forward immediately inferior to the condyles and eventually incline superior-medially to the symphysis. Proximally, the malleus and incus, two of the bones of the middle ear, arise. The next succeeding portion of the cartilage, as far as the lingula, is progressively replaced by fibrous tissue which forms the sphenomandibular ligament. As the cartilage disappears between the lingula and the canine tooth; ossification also proceeds inferior-posteriorly to where the incisor teeth sits. In brief, ossification of the mandibular bone from the Meckel's cartilage is considered to arise independently on the right and on the left side from a single center, which appears near the mental foramen about the fifth to seventh week of gestation. Accessory nuclei of ossification from the cartilage may appear later in prenatal life to bring this complex and multifaceted process forward, but they possess no separate ossification centers. Rather, they are invaded by the surrounding membrane bone, which engenders the process of absorption. By contrast, the inner alveolar border of the

mandibular bone may arise from a separate and distinct ossification center during ontogenesis, the splenial[7] center, which results from an ingrowth from the main mass of the bone. Perinatally, the mandibular bone consists of two parts that are joined by a fibrous symphysis, which completes its process of ossification in the first year postnatally. The condylar process is positioned in the superior-lateral most aspect of the ramus of mandibular bone, which signifies the attachment of three of the four powerful masticatory muscles (i.e., masseter, temporal, medial pterygoid). The condylar and pericondilar aspects of the mandibular bone (e.g., subcondylar region located between the condyle and the coronoid process of the mandible; aka, mandibular notch) are most prone to fracture (36%) during postnatal development and adult life. These are dangerous fractures, which require delicate interventions, because they can engender significant and profound dysfunctions of the temporo-mandibular joint (*vedi infra*), consequential inflammatory events that may precipitate osteoarthritis of the jaw joint. It is also the case, however, and it is important to note that traumatic arthritis without condylar fracture may also develop from indirect transmitted violence to the superior aspect of the ramus of the mandibular bone. From an osteoimmune perspective, condylar fractures are critical. Condylar fractures can be

- Extracapsular
- Subcondylar
- Intracapsular

The powerful lateral pterygoid muscle then tends to cause important anterior and medial displacement of the condylar process (i.e., *processus condyloideus*), which signify the onset of joint disorders, including damage to branches of the trigeminal and facial cranial nerves that run proximally, either lateral or medial, to the condylar and pericondylar aspects. Several types of condylar fractures are consequential to trauma (e.g., accidents, sports), and present in order of increasing severity as follows:

- *Type I*: fracture of the neck of the condyle with relatively slight displacement of the head. The angle between head and axis of ramus can vary from 10 to 45°.
- *Type II*: fracture that produces an angle from 45 to 90°, and that results in tearing of the medial portion of the joint capsule, with potential associated hemarthrosis.
- *Type III*: fracture that is so severe that the fragments are not continuous anymore, but still confined within the area of the glenoidfossa, resulting in significant medial and anterior displacement, and a tearing of the capsule such that the condylar head is now outside the capsule, and associated hemarthrosis. Often, in these cases, the fracture is associated with partial or complete

[7] Derived from splenia (Lt. pl of *spenium*, splint). It is a piece found in the mandibular bone in *homo sapiens*, and well retained throughout phylogeny as it is evident in facial skeletons from dinosaurs, reptiles, birds, and early mammals as well. It serves as a splint typically along the ventromedial surface of the mandible.

rupture of inferior dental artery, which signifies impaired endosteal blood supply and endogenous osteoimmune healing processes.
- *Type IV*: fracture of the condylar head that now comes to articulate on or in a forward position with regard to the articular eminence.
- *Type V*: fracture that presents vertically or obliquely through the head of the condyle.

Hyoid bone rests at the level of the base of the mandible in the front and the third cervical vertebra behind, and provides attachment to the muscles of the floor of the mouth and the tongue above (i.e., Middle pharyngeal constrictor, Hyoglossus, Digastric, Stylohyoid, Geniohyoid, and Mylohyoid muscles), and the larynx below, and the epiglottis and pharynx behind (i.e., Thyrohyoid, Omohyoid, and Sternohyoid muscles). It is a suspended bone held in place by the thyroid ligaments. Thus, it does not articulate with any other, and functions to allow a wider range of tongue, pharyngeal, and laryngeal movements by bracing these structures alongside each other in order to produce variation. Anatomically, it consists of a central part, the body (i.e., *corpus oss. Hyoidei, aka basihyal*), that sits antero-medially, and two pairs of latero-posterior extensions, called the cornua. The greater cornu (i.e., *cornua majora; aka thyrohyals*) project backward from the lateral borders of the body, flatten from above downward, and diminish in size from anterior to backward. They project into a tubercle that attaches the lateral hypothyroid ligament. The lesser cornu (i.e., *cornua minora, aka ceratohyals*) are smaller conical eminences, that are attached by their bases to the angles of junction between the body and greater cornua by fibrous tissue, and occasionally distinct diarthrodial joints (i.e., synchondroses in early ontogeny, and after middle life usually by bony union), which persist throughout ontogeny, but are at risk of become ankylosed (*vedi infra*) in later life. The early ontogeny of the hyoid bone is complex in that the lesser cornu and the most superior aspect of the hyoid body originate from the second pharyngeal arch, while the greater cornu and the lower part of the body of hyoid arise from the third pharyngeal arch. Moreover, ossification of the hyoid bone proceeds from six centers: two for the body, and one for each cornu. The process of ossification commences in the greater cornua during late fetal life, and proceeds rapidly to the body, and terminates in the lesser cornua in the first to second year postnatally. The connection between the body and greater cornu may remain fibrous until middle-age.

1.3.2 The Temporomandibular Joint

The temporomandibular joint (i.e., *articulatio temporomandibularis*; aka, "jaw joint"; TMJ) forms the articulation of the upper temporal bone, which is part of the cranium, superiorly, and the mandibular bone, commonly called the mandible (i.e., the "lower jaw"), inferiorly. The TMJ is a synovial joint, which is nearly unique, with the sternoclavicular joint, in that it possesses an articular disc that

cushions the joint, composed of fibrocartilagenous tissue (i.e., firm and flexible elastic cartilage). From an anatomical perspective, the disc creates two spaces:

- *Inferior* to the articular disc, a TMJ compartment is formed by the mandible and the articular disc, involved in a rotational movement, which is the initial movement of the jaw as the mouth opens. The rotation of the condylar head around its instantaneous axis of rotation permits the first 20 mm or so of the opening of the mouth. Beyond that point, a translational movement[8] is necessary for further extension of the joint, which is achieved by the superior compartment of the TMJ.
- *Superior* to the disc, the TMJ compartment consists of the articular disk and the temporal bone, and is involved in the translational movements of the jaw, which corresponds to the secondary gliding motion of the jaw as the mouth is opened widely. The superior compartment is divided into two parts by a narrow slit, the petrotympanic fissure (Glaserian[9] fissure, named after the Swiss anatomist, Johann Glaser (1629–1675)).

The part of the mandible that conjoins to the under-surface of the disc is the mandibular condyle (*vedi supra*). The aspect of the temporal bone that conjoins with the upper surface of the disk is the glenoid (or mandibular) fossa, a concave depression in the squamous portion of the temporal bone that is bounded anteriorly by the articular tubercle, and posteriorly by the tympanic part of the bone, beyond which lies the external acoustic meatus. That proximity to the auditory organ provides some degree of explanatory rationale for why, as osteoimunnological events precipitate TMJ dysfunctions (*vide infra;* aka temporomandibular dysfunctions, TMD), one common complaint is ringing in the ears (i.e., *tinnitus*[10]). Those relationships develop and establish in ontogenesis around 12 weeks *in utero*, ossification proceeds (*vide supra*), and the joint spaces and the articular disc develops.

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[8] Translation of the TMJ has been traditionally seen as a forward and downward sliding motion, on the anterior concave surface of the glenoid fossa and the posterior convex surface of the articular eminence. However, it is now clear that this translation actually amounts to a rotation around another axis, and that it engenders an "evolute," the resultant axis of mandibular rotation, which lies in the vicinity of the mandibular foramen, allowing for a low-tension environment for the vasculature and innervation of the mandible (Moss 1972).

[9] Not to be mistaken with the term "Gasserian," such as in the gasserian (i.e., trigeminal, aka "semilunar") ganglion, a sensory ganglion of the trigeminal nerve the Meckel's cavity within the dura mater, and that covers the trigeminal impression near the apex of the petrous part of the temporal bone, whence emerge the ophthalmic, maxillary, and mandibular branches of the trigeminal nerve, Cranial nerve V. The gasserian ganglion was named by Anton Hirsh, student of Johann Lorenz Gasser (Austrian anatomist, 1723–1765), in Anton Balthasar Raymund Hirsch. *Pars quinti encephali disquisitio anatomica*. Vienna, 1765. Re-published by Christian Friedrich Ludwig (1751–1823): *Scriptores neurologici minores selecti, sive opera minora ad anatomiam, physiologiam et pathologiam nervorum spectantia*. Volume 1, pp. 244–162. Leipzig, 1791.

[10] To be sure, tinnitus, the perception of ringing or sound within the ear in the absence of corresponding external sound, may result from a range of underlying causes, ranging from ear infections, to foreign objects or wax in the ear, nose allergies that prevent (or induce) fluid drain and cause wax build-up, and side effect of medications, of genetic (congenital) hearing loss, or of noise-induced hearing loss.

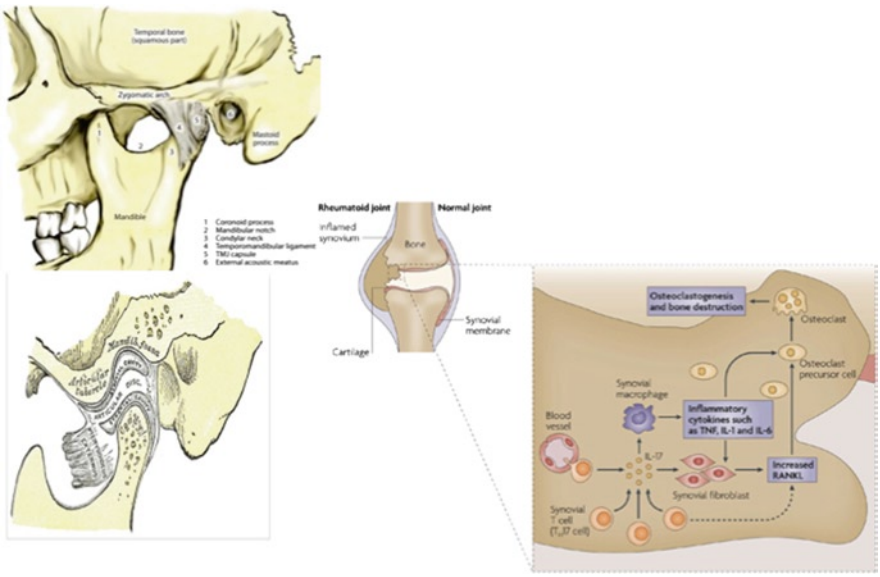

Fig. 1.7 Rheumatoid arthritis in the temporomandibular joint A side and a sagittal view of the temporomandibular-joint are shown, the latter presenting the position of the synovial articular disk between the mandibular condyle and the maxillary articular tubercule. The schematic representation outlines the events leading from inflammation (i.e., IL-17 regulated) to osteoclastogenesis and bone resorption. This process distinguishes the arthrtic joint, with inflammed synovium, from a normal joint characterized by a normal synovial membrane lining. Pathway by which RANKL activates osteoclast bone resorption and synoviocytes, which then invade cartilage is not shown. Takayanagi (2007, 2009); source: http://www.nature.com/nri/journal/v7/fig_tab/nri2062_F2.html

The TMJ is a two-component joint in structure, as outlined above, and function. It is supplied by branches of the external carotid artery, such as the superficial temporal branch, the deep auricular artery, the anterior tympanic artery, the ascending pharyngeal artery, and the maxillary artery. Functionally, the TMJ is a ginglymo-arthrodial joint, and both the articulation in one plane[11] and the gliding[12] properties of the joint can, and will suffer significantly in the case of osteoimmune pathologies, such as osteoarthritis, osteoporosis, or osteopetrosis (*vide infra;* cf. Fig. 1.7). Within these spatial constraints, the TMJ has several movements:

- *Excursions*: normal movements of the mandible during function, such as mastication, or chewing. Here, the working side condyle, which lies on the side

[11] A ginglymal joint is one in which the articular surfaces are molded to each other in such a manner as to permit motion only in one plane.

[12] An arthrodial joint is a synovial joint which, under physiological conditions, allows only gliding movement.

of the mandible that moves outward, performs rotation in the horizontal plane, whereas the balancing side condyle performs translation.
- *Protrusion*: a specific type of nonlateral, but rather forward excursion. Protrusion is accomplished by translation of the condyle down the articular eminence superiorly, without any more than the slightest amount of rotation taking place in the inferior space of the TMJ, other than that necessary to allow the mandibular incisors to come in front of the maxillary incisors without running into them.
- *Retrusion*: a reversal of protrusion.

The physical movements of the TMJ are directed by the four powerful muscles of mastication, and specifically the masseter, medial pterygoid, lateral pterygoid, and temporalis muscles. These muscles are innervated by the mandibular division of the trigeminal nerve (V3) and its sensory branches (i.e., auriculotemporal and masseteric branches of V3), and work to permit the mandible to move in different directions.

1. *Lateral pterygoid* muscle acts to pull the disc and condyle forward within the glenoid fossa, and down the articular eminence (i.e., open the jaw).
2. *Masseter* and *medial pterygoid* muscles close the jaw by pulling up the angle of the mandible.
3. *Temporalis* closes the jaw by pulling up on the coronoid process.

The stability of the TMJ is ensured by fibrous ligaments, which on the one part provide a substantial amount of flexibility and strength, but on the other often harbor inflammatory processes, which may precipitate sustain osteoimmune pathologies that can be significantly damaging to the TMJ and produce a serious impairment in the quality of life of the patient (*vide infra*). Ligaments that define the border movements of the TMJ include:

- The *temporomandibular ligament*, the thickened lateral portion of the capsule composed of an outer oblique and an inner horizontal portion (IHP).
- The *stylomandibular and sphenomandibular ligaments* are accessory and not directly attached to any part of the joint. The former separates the infratemporal region (anterior) from the parotid region (posterior), and runs from the styloid process to the angle of the mandible. The latter runs from the spine of the sphenoid bone to the lingula of mandible.
- *Oto-mandibular ligaments* that connect the middle ear (i.e., malleus) with temporomandibular joint (e.g., discomallear, and malleomandibular ligaments).

1.3.3 Immuno-Biology of Facial Bony Structures

As one considers the immuno-biology of facial bones, the question arises as to whether or not the osteologic structures that constitute the facial skeleton, and which we reviewed above, indeed contain adult stem cell niches, or are at all endowed with significantly relevant extent bone regeneration. Understandably, that question is clinically relevant for oral surgeons, implantologists, and oral biologists in general.

Research findings over the past few years converge to the widely accepted view that, in fact, bone marrow stem cells have the potential to recreate tissues of the craniofacial region to restore normal structure and function in reconstructing the hard tissues of a face (Robey and Bianco 2006). Indeed, postnatal skeletal stem cells are a subpopulation of the bone marrow stromal cell network, and in fact only 10%, at most, of the bone marrow clonal strains can form bone as definite adult skeletal stem cells (Bianco et al. 2006).

Whereas they are fundamentally similar to other bones, the osteological structures that constitute the splanchnocranium demonstrate discrete responses to developmental, mechanical, and homeostatic regulatory signals. Adult bone marrow stem cells obtained from either the mandible or long bones differentiated into osteoblasts, but mandible-derived cells exhibited a distinct epigenetic program, as manifested by increased expression of specific osteoblastic proteomic signature (e.g., alkaline phosphatase activity, mineralization, osteoblast gene expression), and overall greater effectiveness in the formation of colonies and larger bone nodules containing significantly more mineralized bone compared to osteoblasts derived from long-bone bone marrow stem cells. Taken together, the data to date suggest that mandible bone marrow-derived stem cells are endowed with increased osteogenic potential and augmented capacity to induce bone formation in vitro and in vivo, compared with the potential of bone marrow-derived osteoblasts obtained from any long bone of the articular skeleton (Aghaloo et al. 2010).

Case in point, in situations of bone atrophy and pneumatization of the maxillary sinus consequential to loss of teeth in the posterior maxilla, the dimension of alveolar ridge is decreased and sinus augmentation procedures are to create bone quantity and quality to ensure the placement of dental implants (Park 2010). Failure to provide adequate support at the posterior dentition will lead to a series of occlusion issues, including loss of vertical dimension of the temporomandibular joint (TMJ), with possible compression of the auriculo-temporal nerve and local trigeminalgia, as well as general trigeminalgia via the Gasserian ganglion (Demerjian et al. 2010). The fact that adult stem cells, derived from various tissues including bone marrow, periosteum, and trabecular bone, have been successful in sinus augmentation procedures both experimentally and clinically across the board (Park 2010), together with the observation that bone morphogenetic proteins (*vide infra*, BMPs) manifests osteoinductive properties in this context (Park 2009), attests to the osteobiological and osteoimmune resiliency of the facial skeleton.

As discussed earlier, BMPs and osteogenic proteins (OPs) are pleiotropic members of the transforming growth factor (TGF)-β supergene family, and as such play a central role in the osteoimmune network discussed here. These proteins act as soluble signals for the de novo initiation of bone formation, for sculpting the multicellular mineralized structures of the bone–bone marrow organ, and, in the context of the facial skeleton, not only favor the induction of cementogenesis, but also induce the morphogenesis of the periodontal ligament system with a faithful insertion of Sharpey's fibers into the newly formed cementum. In the facial bony structure, as in the skeleton in general, these OPs of the TGF-β superfamily contribute to sculpting tissue constructs that engineer skeletal tissue regeneration in molecular terms:

in brief, they regulate the induction of bone, such that, as bone develops a mosaic structure, cytokine members of the TGF-β superfamily singly, synergistically, and synchronously initiate and maintain tissue induction and morphogenesis (Ripamonti et al. 2005). Direct translational implications of these basic findings of fundamental oestoimmunology is the clinical observation that clefts of the anterior maxilla can undergo complete osseous regeneration induced by recombinant human bone morphogenetic protein type-2 (rhuBMP-2) (Herford et al. 2007).

Furthermore, distraction osteogenesis is a fundamental event of the cranio-maxillo-facial reconstruction process. The molecular mechanisms that regulate bone synthesis in the interfragmentary gap resulting from the gradual separation of bone segments, which signify the fundamental histology and cytology associated with distraction osteogenesis, are driven in large part by the RANK/RANKL/OPG system, discussed earlier (*vide supra*) for its central role in regulating bone metabolism and osteoclastogenesis. Taken together, the data to date strongly support an important biological and molecular role for the influence of the RANK/RANKL/OPG system on the bone healing and remodeling processes in distraction osteogenesis, and suggest possibly developing molecular interventions directed at improving the clinical outcome for distraction osteogenesis[13] (Pérez-Sayáns et al. 2010).

[13] Distraction osteogenesis, a surgical intervention introduced by the Italian surgeon Alessandro Codivilla (1861–1912) is used to reconstruct skeletal deformities primarily of the long bones, and is also applied to correct deformities of the maxillary and mandibular bones. When successful, the intervention simultaneously increases bone length and the volume of surrounding soft tissues. Maxillofacial surgeons use distraction osteogenesis for the correction of micrognathia, midface, and fronto-orbital hypoplasia in patients with craniofacial deformities.