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Fracture healing: From basic science to role of nutrition

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TABLE OF CONTENTS

1. ABSTRACT

Fracture healing is a complex event that involves the coordination of different processes: initial inflammatory response, soft and hard callus formation, initial bony union and bone remodeling. This well-orchestrated series of biological events follows a specific temporal and spatial sequence that can be affected by biological factors, such as age and bone quality. There is some evidence that increased age is a considerable factor in the inhibition of fracture repair in human subjects. During aging there is an accumulation of damage that depends on the activation of inflammation processes and on changes in the circulating levels of inflammatory cytokines. In addition to the physiological slow down in the repair process, other conditions such as multiple comorbidities leading to polymedication are a frequent occurrence in elderly patients and can have an influence on this process. A further factor that affects bone metabolism is nutrition: bone quality, fragility fractures risk and fracture healing process are all influenced by the nutritional status. This review provides a summary of the immunological aspects of physiological fracture healing and of those nutritional factors which might play an important role in this process.

2. INTRODUCTION

Fracture repair remains to a great extent an unknown cascade of biological events made by a complex and highly regulated process influenced by physiological, cellular and molecular/genetic factors (1). Many factors play a role in this process including growth factors, inflammatory cytokines, antioxidants, bone resorption (osteoclast) and bone formation (osteoblast) cells, hormones, amino acids, and uncounted nutrients (2). A fracture is associated with the disruption of the bone integrity, the interruption of vascular function and a distortion of the bone marrow architecture; like all other wound repair responses, fracture healing is initiated through the induction of an innate immune response (3,4). Degranulating platelets, macrophages, and other inflammatory cells (granulocytes, lymphocytes, and monocytes) infiltrate the hematoma in the fractured fragments, prevent infection, secrete cytokines and growth factors, and advance clotting into a fibrin clot (5,6). The inflammatory response is the result of both local tissue injury and immunologic reaction that is caused by local necrosis, bacterial entry and hypoxia. The innate and adaptive immune responses provide protection against

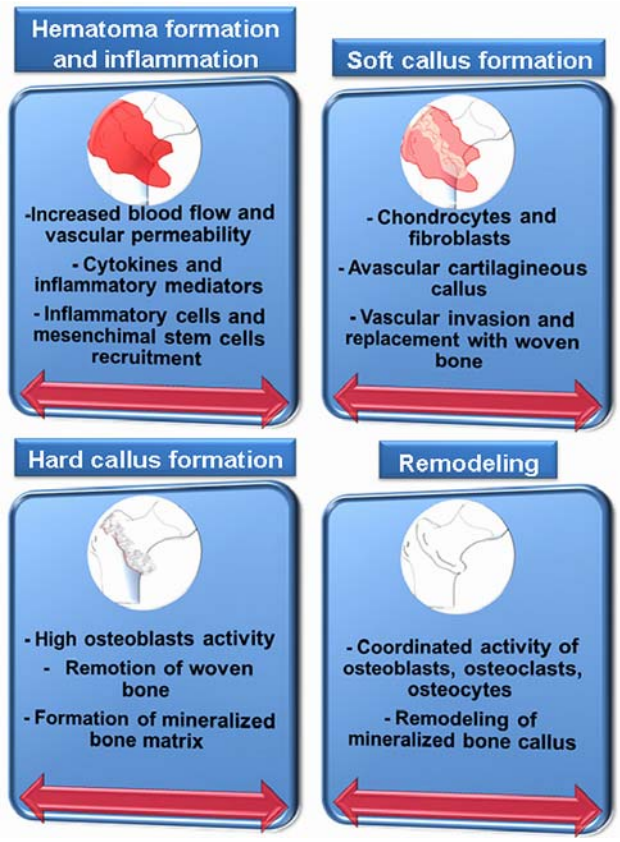

Figure 1. Fracture healing phases and events.

specific pathogens and produce inflammatory reactions responsible for host defense in pathological conditions. Lastly, fibroblasts migrate into the fracture site and begin to transform the hematoma into granulation tissue; after this event a network of new capillaries stimulated by various cytokines invades the granulation tissue (7,8). In geriatric patients the impairment of fracture healing process is due to the comorbidities occurring in this age such as diabetes mellitus, anaemia, moderate or severe functional alterations and osteoporosis. In addition, malnutrition is an important geriatric problem and dietary factors influence the physiological pathways of aging and life expectancy. Several nutritional factors such as vitamin D, calcium and antioxidants play an important role to prevent bone loss and the impaired fracture healing in elderly (9,10). The aim of this review is to summarize the immunological aspects of physiological fracture healing with particular regard for molecular and cellular interactions, and to highlight the importance of some nutritional factors on bone health.

3. BONE FRACTURE HEALING

Fracture healing depends on a complex interaction of cells, biologic pathways, and molecules that interact in space and time to produce a response to a bone loss of integrity. This complex cascade of biological events involves intracellular and extracellular molecular signaling for bone induction and conduction (11,12).During fracture healing the molecular mechanisms that regulate the skeletal tissue formation during embryological development are recapitulated with the coordinated participation of several cell types (13,14). After a bone fracture a complex sequence of events is set in motion resulting in restoration of continuity and strength. At the end of the healing process, the resulting tissue is similar both microscopically and macroscopically to the bony tissue present before the injury; this process is unique in the body, indeed the tissue ultimately heals without scarring. Many local and systemic regulatory factors, including growth and differentiation factors, hormones, cytokines, and extracellular matrix, interact with several cell types, including bone and cartilage forming primary cells or even muscle mesenchymal cells, recruited at the fracture-injury site or from the circulation. Two basic histological types of bone healing are described: primary and secondary bone healing. Primary healing refers to a direct attempt of the cells in cortical bone to re-establish the disrupted continuity. It is rare and requires absolute contact of the fragments and almost complete stability (15). Secondary bone healing occurs in the great majority of bony injuries, involves both intramembranous and endochondral ossification and leads to callus formation (7). The characteristic sequence of events, originally described by McKibbin, are hematoma, inflammation, formation of soft and then hard callus and finally remodeling (16,5) (Figure 1). Several researches in the past decades have explored both the cellular and molecular forces that drive the underlying processes

(13,17,18). In the repair process, the key players at the cellular level are inflammatory and vascular cells, osteochondral progenitors, osteoblasts, osteocytes and osteoclasts. At the molecular level, fracture repair is driven by the three main classes of factors: pro-inflammatory cytokines and growth factors, pro-osteogenic factors, and angiogenic factors (4).

A fracture is associated with the disruption of the local tissue integrity, the interruption of the normal vascular function and the distortion of the marrow architecture. The release of inflammatory mediators from the fracture hematoma initiates a local inflammatory reaction that is the same response that occurs in others injured tissues: it is characterized by an increased blood flow and vascular permeability, the migration of inflammatory cells and the further release of cytokines and activation of the complement cascade (19,20).The soft callus formation is dominated on a cellular level by the vascular invasion (15). This cartilaginous callus is initially avascular but its subsequent replacement with woven bone involves vascular invasion (21-23). The hard callus formation represents the most active stage of osteogenesis and it is characterized by high levels of osteoblastic activity and by the formation of mineralized bone matrix (24). In order to bridge new hard callus, the temporary soft callus is gradually removed at the same time of revascularization (25,26). The formation of new blood vessels is critical for the subsequent hard callus, producing locally increased oxygen tension necessary for osteoblast differentiation. Bone remodeling, the final stage of fracture repair, results from a coordinated activity of three cell types: bone forming cells or osteoblasts, their final stage of differentiation termed osteocytes, and the bone-resorbing cells or osteoclasts. Osteoblasts differentiate from multipotent stromal cells similarly to chondrocytes, adipocytes and myocytes. During fracture healing fibroblastic cells and preosteoblasts produce type III collagen that forms a moderately dense fibrous tissue that replaces the initial hematoma (27); then type I collagen replaces this tissue and forms the mature bone matrix. As a matter of fact, mature osteoblasts synthesize and secrete large amounts of type I collagen, non-collagenous proteins, enzymes and growth factors that comprise major components of the organic matrix of bone (osteoid) that later become mineralized (28).

Osteoblasts are critical regulators of the hematopoietic stem cells and they secrete two different cytokines essential for the induction, survival and competency of osteoclasts: Macrophage colony-stimulating factor (M-CSF) and Receptor Activator of NF-kB Ligand (RANKL) (29). M-CSF induces the hematopoietic stem cell differentiation towards osteoclasts and it is involved in stimulating the replication of osteoclast progenitors. Mature osteoblats produce RANKL which governs bone formation and bone resorption and it is responsible for stimulating osteoclasts differentiation. RANKL is a member of the tumor necrosis factor (TNF) family of cytokines and plays a key role in osteoclastogenesis and thus inflammatory bone loss (30-33).

While on the one hand, in past times osteocytes appeared as static and inactive cells with limited physiological and pathological interest on the other hand today they are considered as cells with a very active role in bone metabolism (34). They are old osteoblasts embedded into mineralized matrix, they represent the most abundant cells type of bone and they are interconnected by cellular extensions going through a network of canalicules that detect changes affecting the bone (35-37). Osteocytes respond to mechanical stimuli by producing and secreting several molecules, such as nitric oxide and prostaglandin E2 that initiate local bone remodeling; moreover, they can control bone formation by modulating the WNT signaling pathway (38). WNT signaling promotes the expression of osteoprotegerin (OPG) but it inhibits the expression of high levels of osteocalcin, a typical feature of mature matrix-synthesizing cells (29). Osteocytes also represent the main source of sclerostin, a specific molecule that blocks bone formation. Low sclerostin expression leads to bone growth, whereas high expression inhibits bone formation; recently, TNF-α has been identified as an inducer of sclerostin expression (39). Sclerostin inhibits WNT signaling with a negative feedback mechanism. The identification of sclerostin and the demonstration that its expression in the bony tissue is restricted primarily to the osteocytes, provided the first functional evidence that osteocytes directly control bone formation (40).

Recently, genetic studies in mice have revealed that osteocytes provide the majority of the RANKL that controls osteoclast formation in cancellous bone (41,42). They also highly express osteoprotegerin (OPG) and most of it appears to be trapped by RANKL on the surface of osteocytes (43).

Unlike short-lived osteoblasts, osteocytes are long-living cells and their death is dependent on skeletal age, sex or altered bone turnover. Although the death of osteocytes induces bone resorption, their physiological function has been considered to be the inhibition of bone resorption (44,45).Osteoclasts are a large multinucleated bone-specialized cells that differentiate from hematopoietic precursors along the monocytic lineage, they are the only cells of the organism able to reabsorb bone and when activated begin the resorption of bone debris at the fracture site (24,46). Osteoclasts are also cells with a relatively short life and they have to be continuously generated at or near the site of bone resorption. Support cells provide the production of differentiation and survival factors that govern the generation of osteoclasts at specific sites (47). Osteoclastic activity is controlled by factors which drive the fusion of mononuclear precursors in order to form mature multinucleated osteoclasts and one of these factors is RANKL (48). TNF-α was shown to stimulate RANKL-induced osteoclastogenesis by increasing the level of RANK expression (46). OPG is a secreted decoy receptor that is an important regulator of RANKL signaling and antagonizes osteoclast differentiation (49). The relative concentrations of RANKL and OPG determine the extent of proliferation and differentiation of osteoclast precursors in bone and therefore bone mass. Most of the factors that stimulate osteoclast formation exert their effects indirectly by stimulating expression of RANKL by osteoblastic cells, including parathyroid hormone (PTH), interleukin (IL)-1,

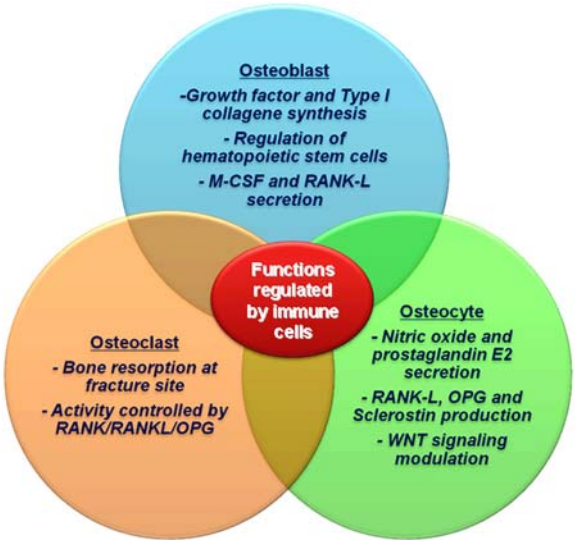

Figure 2. Bone cells and their role in fracture healing.

TNF-α and numerous other hormones, cytokines, and growth factors (50). Osteoclast differentiation and function are also regulated by immune cells such as T and B lymphocytes which produce RANKL and⁄or M-CSF and therefore can stimulate osteoclast formation in inflammatory conditions affecting the skeleton (51). B cells may also have a dual role in osteoclastogenesis, stimulating it through an increased production of RANKL or inhibiting it through increased expression of OPG (52,53) (Figure 2). Theoretically, the absence of osteoclast activity occurring after skeletal maturity could be associated to a poor bone quality and to an increased risk of fractures (54).

4. FRAGILITY FRACTURES

The observation of the demographic trend over the last years has shown an increase in life expectancy, associated with a greater frailty of elderly people and a higher prevalence of chronic and degenerative diseases, including osteoporosis (55,56).

Osteoporosis is characterized by an impairment of both structural properties and bone quality and it predisposes the patient to an increased risk of fragility fractures. Although bone mineral density, measured by DEXA, remains the best available non-invasive assessment of bone strength in routine clinical practice, many other skeletal characteristics also contribute to bone strength. These include bone architecture, matrix and mineral composition, as well as the degree of mineralization, microdamage accumulation and the rate of bone turnover that can affect the structural and material properties of bone (57) (Figure 3).

Osteoporotic fractures are an important public health issue in the aging population and impose a major economic burden worldwide. They can lead to consequences such as hospitalization, long periods of immobility, need of surgical treatment, increased disability and partial or complete loss of autonomy in daily activities (58). Genetic components influence the risk of osteoporosis such as hormonal and nutritional factors and exercise (59,60). Several researches have shown a strong association between aging and osteoporosis, this representing a serious issue due to a marked increase in life expectancy (55). Osteoporosis has generally been considered a women's disease; this may explain why this pathology has focused less attention on men. It has been underestimated and poorly treated in female patients, the situation is even worse in male patients, despite the fact that up to one third of hip fractures are suffered by men (61). The differences between sexes in the bone involution make male individuals more resistant to fragility fractures, therefore generally suffering these fractures later in life than women (62). As age increases, bone mass decreases as a result of changes in the circulating levels of specific hormones (eg. decreased estrogens levels after menopause) and of the reduced anabolic effects of mechanical loading (eg. decreased physical activity). Up to 70% of bone turnover and resorption appear to be modulated by estrogens and 30% by testosterone, so both hormones have an important role in bone formation mechanisms (63,64). The estrogens deficiency after menopause results in an imbalance with a substantial increase in bone turnover, this leading to a progressive loss of trabecular bone, partly due to an increase of osteoclastogenesis (65).

It has been documented that osteocytes density declines with aging and their apoptosis increases in women with estrogens deficiency; the loss of osteocytes reduces bone mass and compromises bone quality with trabecular microstructural alterations, intracortical porosity and microfractures suggesting that osteocytes deficiency/malfunction may underlie bone fragility under various conditions (66,67). The increase of functional osteoclasts appears to be the result of increased elaboration of osteoclastogenic proinflammatory cytokines which are negatively regulated by estrogens (68). Animal studies on ovariectomized fractured mice have shown how estrogens deficiency negatively affects all stages of fracture healing,

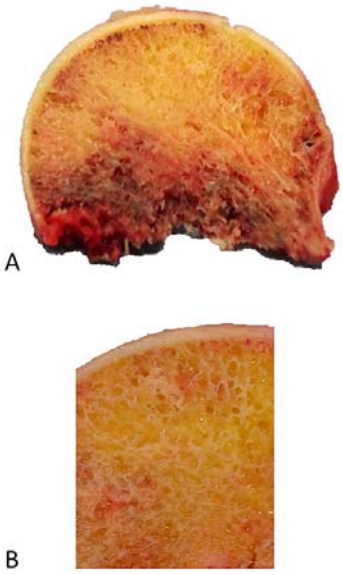

Figure 3. Osteoporotic bone tissue from the femoral head of a fractured patient. A - Femoral head B - High magnification.

particularly the mineralization and remodeling phases. These results confirm that estrogens deficiency in post-menopausal women could be an important factor in the development of non-unions and delayed fracture healing (69). The fracture risk is increased in osteoporotic patients as a result of alterations in bone remodeling, where osteoclastic bone resorption exceeds osteoblastic bone formation; this imbalance causes a loss of bone mass (70). The skeletal sites most commonly interested by a fragility fracture are: femoral neck, proximal humerus, vertebrae, wrist and ankle. It must then be considered how the presence of a fragility fracture represents the major risk factor for a subsequent fracture, with rates increasing from 2 to 5 times (71).

A recent study, based on a 3-year multicenter survey, has estimated an annual incidence of 410,000 new hip, humeral, wrist, ankle, and vertebral fragility fractures in Italy. Considering that Italy represents one of the countries with the highest life expectancies in the world, these data could anticipate possible demographic scenarios of other European industrialized countries (72). Furthermore osteoporosis changes in bone metabolism could negatively affect fracture repair, leading to a "physiologically impaired fracture healing". The decline in the capacity for fracture repair has been shown to be age related but the real pathway by which these alterations influence bone-healing progression, remains still unclear (73). Clinical experience is inconsistent regarding whether bone healing is delayed in the presence of osteoporosis. Too few studies are available on the differences between normal and osteoporotic healing to suggest that there is a reduced capacity of osteoporotic bone to repair. Experimental studies documented cellular alterations in osteoporotic conditions including reduced number of mesenchymal stem cells (MSCs) with consequent gradual replacement of red marrow by adipose tissue, impaired ability of MSC response to humoral stimuli (with a reduced proliferation capacity and osteogenic differentiation), reduced osteoblastic response to mechanical stimuli (lower production of TGF-β, resulting in a decrease of fibroblasts, chondroblasts and osteoblasts proliferation). Moreover, in senile osteoporosis, it has been established that MSCs tend to differentiate towards adipose tissue, with consequent reduction in osteogenesis.

The interaction between the immune and the skeletal systems has long been acknowledged; it has been recognized that T lymphocytes and their products are key regulators for bone remodeling (74). The regulatory interactions between T lymphocytes and bone cells are regulated by molecular mechanisms, various cytokines, and signaling transducers (75,76). T cells govern osteoclastogenesis by secreting several cytokines such as IL-1, IL-6, interferon (IFN)-γ or IL-4 (77,78). T lymphocytes and bone cells also share a common site of origin, the bone marrow that hosts fully functional and mature T cells that exhibit several distinctive features. In the bone marrow mature T cells represent about 3%-8% of the total nucleated cells, they contribute to the homeostasis of the immune system and of the bone cells present in bone marrow environment (79). Activated T cells may undermine bone homeostasis and stimulate bone destruction in pathological conditions such as estrogens deficiency that causes osteoporosis by increasing T cell activation-induced proliferation and suppressing the apoptosis of active T cells (80). Different studies on post-menopausal bone loss have demonstrated that women with post-menopausal osteoporosis have an higher T cell activity compared to healthy post-menopausal subjects (81). Among T cells, T helper cell 17 (Th17) is important in inducing bone loss (82).

Recent experimental studies in elderly rats showed that systemic and local impairment of the inflammatory response leads to delayed fracture healing. Although a local and well controlled inflammation seems to be essential for bone repair, increased or prolonged local and systemic inflammation negatively affects fracture healing. Systemic inflammation has shown to induce hypertrophic and immature callus formation, with reduced bone mechanical properties (69). It has been found that the alteration of signaling molecules such as cytokines IL-1, IL-6 and TNF-α, and such as growth, differentiation and systemic factors contribute to the impairment of the healing process with mechanisms that are not yet clearly understood. It is believed that in osteoporotic bone there is a reduced response in the osteoblastic activity due to impaired influence and the presence of these factors (83).

5. PHYSIOLOGICAL INFLAMMATORY PROCESS AND IMPAIRMENT IN OSTEOPOROTIC PATIENT

In the bone marrow hematopoietic and immune cells are involved in the coordinated participation to the fracture healing, in conjunction with vascular and skeletal cell precursors that are recruited from the surrounding tissues and the circulation. Bone cells interact with immune

cells and play an essential role in the development of the bone marrow space during growth and fracture healing (84). Inside bone and in the surrounding soft tissue the fracture leads to blood vessel rupture, it damages other cells and tissues and it also promotes the inflammatory cascade and fracture healing (85). The environment takes on usual characteristics of acute inflammation, with vasodilatation and exudation of plasma and leukocytes and the fibrinogen is converted into fibrin, leading to fracture hematoma formation (86,87). The inflammatory response initiates bone healing, osteoprogenitor cells, mesenchymal cells, osteoblasts and chondrocytes contribute to the healing leading to the hard callus formation (88,89). The fracture hematoma has been proven to be a source of signaling molecules that may induce a cascade of cellular events that initiate healing. These factors are secreted by endothelial cells, platelets, macrophages, monocytes, but also by MSCs, chondrocytes, osteocytes and osteoblasts themselves (90). IL-1, IL-6 and TNF-α are known to play a role in initiating the repair cascade (91). These cytokines carry out central functions in the responses to injury, they have chemotactic effect on other inflammatory cells, enhance extracellular matrix synthesis, stimulate angiogenesis and recruiting of fibrogenic cells to the site of injury (5). Their serum concentration increases within the first 24 hours following fracture, however, their levels are reduced during the stage of cartilage formation before increasing for a second time during the bone remodeling phase (92). Though playing a positive role during healing, inflammation is also associated with perturbations to healing and age-related changes in the inflammatory system are associated with decreased healing potential (93). During aging there is an accumulation of damages which ensues from chronic activation of inflammation processes; circulating levels of pro-inflammatory cytokines are elevated in older subjects and have been linked to a number of conditions (94).

6. MOLECULAR COOPERATION

Several cytokines are known to be involved in bone remodeling through the regulation of immune function and inflammation. IL-1 is a strong peptide stimulator of bone resorption and its effects seem to be both direct on osteoclasts and indirect through the stimulation of RANKL production (95,96). It increases prostaglandin synthesis in bone which may account for some of its resorptive activity because prostaglandins are also a potent resorption stimulus (97). A natural inhibitor of IL-1, IL-1 receptor antagonist (IL-1ra), is an analog of IL-1 that binds but does not activate the biologically important type I IL-1 receptors. The interaction between IL-1 and its receptors is prevented by IL-1ra and thus it competitively inhibits the biological effects of IL-1. The main source of IL-1 during this inflammatory phase are macrophages (98). IL-1 stimulates angiogenesis and promotes formation of the cartilaginous callus that stabilizes the fracture site; it is expressed by osteoblasts and facilitates bone remodeling by stimulating proteases to degrade callus tissue (99,5). IL-6 is expressed and secreted by cells of the osteoblastic lineage and osteoclasts in response to parathyroid hormone, vitamin D and IL-8 (100). IL-6 has been shown to participate in the early stages of fracture repair, it's produced in response to stimulation by IL-1 and promotes angiogenesis by stimulating release of vascular endothelial growth factor (101). IL-6 promotes osteoclast activation and the resulting bone resorption through the RANK/RANKL/OPG pathway; it induces differentiation of pre-osteoblasts to mature osteoblasts while it decreases the proliferation of osteoblasts at late differentiation stages. IL-6 serum levels remain at baseline in the remodeling phase of fracture healing. Murine models of fracture healing have shown that IL-6 ablation alters early fracture healing (102). TNF-α might participate in the induction of early inflammatory response; it is a primary mediator of immune regulation, an important component of almost all inflammatory responses, and is produced by a wide variety of immune and non-immune cells. TNF-α has been studied in bone and cartilage metabolism and it has been implicated in osteoclastogenesis (103). TNF-α serum levels have been shown to be elevated in surgical or natural menopausal states but markedly reduced during the stage of cartilage formation thus implicating its involvement in control of bone turnover; the increase of TNF-α concentration could be an important risk factor for fracture (91).

IL-6 and TNF-α serum concentrations are different in young fractured patients and elderly fractured patients underlining the impairment of the healing process in osteoporotic fractured patients (92). The RANKL has been shown to be a key regulator of osteoclastogenesis; it has a strong activity as a stimulator of both formation of osteoclasts from precursor cells and bone-resorbing activity in mature osteoclasts (104). In inflamed sites, immune cells and bone cells share the microenvironment: the key effectors in this microenvironment include the triad of RANKL, RANK, and OPG molecules. Pro-inflammatory cytokines can modulate the RANKL, RANK and OPG system, the RANKL-RANK axis is essential for osteoclastogenesis (105,106). The balance between RANKL/RANK and OPG has direct effects on bone loss in some diseases, such as osteoporosis, chronic inflammatory arthritis and the osteolytic bony metastasis of malignancies. Targeted disruption of RANKL results in defective formation of the lymph nodes and lymphocytes differentiation, as well as osteopetrosis, a sclerotic bone disease caused by impaired osteoclastic bone resorption (107). The extensive distribution of RANKL throughout the body already indicates its multiple functions, whereas the most important one is dedicated to the induction of osteoclastogenesis and therefore to the regulation of bone remodeling (108). RANKL knock-out mices reveal a severe osteopetrotic phenotype due to the absence of osteoclasts. The interferon-γ (IFN-γ) was identified to strongly suppress osteoclastogenesis by inhibiting RANKL signaling (109). INF-γ is also reported to stimulate resorption through enhanced RANKL and TNF-α production in T lymphocytes. Moreover, it inhibits osteoblast proliferation and has variable effects on osteoblast differentiation.

OPG is a potent inhibitor of osteoclast formation that acts as a decoy receptor for RANKL; it was initially identified as a soluble factor capable of inhibiting osteoclastogenesis *in vitro* (110) and terminal stages of

osteoclast differentiation. OPG binds the RANKL with an high specificity preventing osteoclasts differentiation and activation, and promoting osteoclasts apoptosis. Therefore, the balance between RANKL and OPG determines bone resorption (111). Most studies found elevated OPG serum levels in patients with osteoporosis and during fracture healing the OPG concentration seems to be higher in osteoporotic fractured patients than in young fractured patients (92). IL-17 induces the synthesis of matrix-degrading enzymes, such as matrix metalloproteinases, inducing bone and cartilage degradation; it also stimulates the local production of RANKL by inflamed tissues and produces RANKL that enhances resorptive destruction of bone at sites adjacent to the inflammation (97). Inhibition of IL-17A reduces bone destruction. IL-23, a recently discovered cytokine, can stimulate osteoclast formation in two independent pathways: by up-regulating RANKL expression in osteoblasts and by acting on myeloid precursors inducing the RANK B expression (112). The IL-23/IL-17 axis has been demonstrated to be a potential linker between inflammation and bone loss. Insulin-like growth factor1(IGF-1) is secreted both by chondrocytes and osteoblasts and it is an important factor promoting osteoblastic activity (113). IGF-1 levels increased after the fracture and reached maximal levels in the middle to late stage of callus formation. IGF-1 concentration significantly declines with age in both men and women, especially in osteoporotic individuals with very low bone density; this is most likely due to a reduction in growth hormone secretion (114).

IL-4 is a so-called ‘‘inhibitory cytokine’’, a term used by immunologists to describe molecules that counteract the proinflammatory effects of TNF-α and IL-1. IL-4 targets both osteoclasts and osteoblasts by inhibiting *in vivo* bone remodeling (115). The inflammatory process during fracture repair is evidently modulated by different molecules; in osteoporotic patients this well orchestrated series of biological events changes and the cross-talk mechanisms between skeletal and immune system are modified.

7. NUTRITIONAL FACTORS AND BONE QUALITY

Aging is often accompanied by malnutrition or undernutrition. Deficiency of nutritional factors appears to be strongly implicated in fracture in the osteoporotic elderly. Nutritional personalized programs could prevent bone loss and thus fragility fractures in aged people. Many compounds in food and plants act on bone metabolism, stimulate osteoblastogenesis, inhibit osteoclastogenesis and reduce inflammatory condition (116,117). Oxidative stress play an important role in pathophysiology of the aging process: oxidative stress is an imbalance between free radicals and antioxidant mechanism and it damages macromolecules and cellular functions. When bone fracture occurs the damaged tissue increases free radicals, similarly to what occurs when osteoclastic activity increases. Free radicals are produced also in response to inflammatory stimuli and TNF-α increases intracellular oxidative stress (118). Oxidative stress is associated with osteoporosis and may be reduced by dietary antioxidant. Polyphenols have a positive role in the prevention of cardiovascular diseases, cancer and osteoporosis. As a matter of fact they act by increasing trabecular bone volume and bone mass, enhancing bone formation and inhibiting bone resorption (119-123). Among dietary antioxidants, lycopene reduces the levels of bone turnover markers and the risk of osteoporosis; carotenoids are important in bone remodeling, since they reduce fracture risk decreasing bone resorption and increasing bone formation; vitamin C inhibits the differentiation of precursor cells in mature osteoclasts and reduces the bone resorption (124,125).Vitamin C has also been shown to be essential for the maintenance of differentiated functions of osteoblasts, including those in fracture repair (126). Indeed, it has been demonstrated that vitamin C supplementation accelerates fracture healing in an animal model (127).

Although necessary for bone development, vitamin A has negative effects in case of high intake; this leads to an increased incidence of hip fractures and it is associated with poor bone quality (128). Therefore the balance between carotenoids and vitamin A is important for a proper bone development. In elderly subjects vitamin’s deficiency is common, particularly in osteoporotic patients and the improvement of their intake may help to treat and prevent osteoporosis. An increase in administration of dietetic antioxidants could protect bone from osteoporosis and could lead to reduction in fractures with personal and social benefits. In addition antioxidants could help in the acceleration of fracture healing (125). Fatty acids have beneficial effects in several diseases including osteoporosis (129-132). Their action mechanism is based on anti-inflammatory effects through the decrease in production of pro-inflammatory cytokines like TNF-α, IL-1β and IL-6 (133). *In vivo* and *in vitro* studies have shown that the dietary fatty acids may play an important role in enhancing bone formation and suppressing the production of bone absorption (134,135). High concentration of isoflavones, including genistein, are contained in soyben. Isoflavones have been demonstrated to have anabolic effect on bone metabolism and to prevent osteoporosis. They play a role in protein synthesis and gene expression in relation to bone formation and bone resorption (136). The anabolic effect may be correlated to the binding of estrogens receptor β in osteoblastic cells (137). Isoflavones differ in the chemical structure from other flavonoids: the B-C rings bond in the C3 position instead of the C2 position and conferring them a pseudohormonal activity because it resembles the human hormone 17-beta-estradiol. This compounds are classified as phyto-estrogens (10). Isoflavones have a potential role to prevent bone loss in aging; oral administration of soybean extract has been demonstrate to increase bone components in rats, confirming an anabolic effect on bone metabolism (138). The alkaline phosphatase’s activity, a marker enzyme in differentiation of osteoblastic cells, increases in presence of genistein.

Zinc, an essential trace element, is involved in the differentiation of osteoblastic and osteoclastic cells, and is required for growth, development and maintenance of bone health (139). It could be possible that zinc increases the concentration of OPG in osteoblastic cells. During

fracture repair the presence of zinc increases alkaline phosphatase activity and stimulates osteocalcin production; therefore zinc supplementation may promote fracture healing (140). In osteoporotic subjects the level of skeletal zinc is lower than in controls. The combination of zinc and genistein has a synergistic effects on osteoblastic cells, it enhances bone mineralization and increases bone mass (141). Calcium (Ca) is an essential structural component of bone, its levels are correlated to bone mineral density (BMD) and to the risk of osteoporosis and fragility fractures. A daily consume of dairy products maintains bone health and reduces osteoporosis. Dairy products may represent the best dietary sources of Ca due to their high calcium and nutrients content. Vitamin D is involved in the control of blood calcium levels and its insufficiency is common in elderly people (142,143); this condition may lead to bone loss and it increases the risk of fragility fractures. The National Osteoporosis Guideline Group (NOGG) recommends a daily intake of at least 1000 mg of calcium, 800 U of vitamin D, and 1 g/kg body weight of protein as a general measure for osteoporosis prevention. The nutritional factors appear certainly implicated in bone health, and they play an important role in osteoporosis and fracture healing process, enhancing bone formation and reducing bone absorption.

8. CONCLUDING REMARKS

Fracture repair is a complex and highly regulated process that is influenced by physiological, cellular, and molecular/genetic factors. The osteoimmunological approach has revealed a new perspective to understand fracture healing process, demonstrating that activated lymphocytes can contribute to the changes in bone remodeling. The crosstalk is not only within the immune and the musculoskeletal systems but there is also a bidirectional crosstalk between the two systems. All immune cells are able to communicate with osteoblasts, osteoclasts and their respective progenitors and vice versa. Furthermore they could also be involved in the bone extracellular matrix remodeling. During inflammation immune cells and bone cells also share each other's microenvironment and the consequence of this conversation is usually destructive. Pro-inflammatory cytokines released from activated T-cells target osteogenic cells and change their expression profile causing the further production of pro-inflammatory cytokines and chemokines, the up regulation of RANKL and stimulate the activity of matrix metalloproteinases in osteogenic cells. Cytokines exhibited their osteoclastogenesis-related activity only when a permissive level of RANKL exists. In conclusion, various components of the inflammatory response can either stimulate or compromise healing through the regulation of cell recruitment and differentiation. An early hyperinflammatory local reaction might be related to the initiation of the fracture healing process. More molecular and cellular studies are needed to understand the changes of the different microenvironments in the bony tissue that has a specific tridimensional organization of extracellular matrix. Further researches will have to identify the factors that are incompatible with fast and successful fracture repair and will have to find methods to replace the unfavorable with favorable factors. These efforts will hopefully result in an improved care of patients with problematic fractures, including the osteoporotic ones. Nutrition is an important adjunctive therapy for osteoporosis and many nutritional factors have a positive effect on fracture healing; adequate dietary intakes of bone nutrients reduce the risk of osteoporosis and fracture in elderly life. A nutritional approach could be a way to improve bone health and to reduce fracture risk.

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