Chapter

Role of Inflammatory Factors in Regulation of Osteogenesis in Tissue-Engineered Bone

Yandong Mu, Lu Yang, Chenglong Li and Wei Qing

Abstract

It was traditionally considered that the inhibition of inflammatory reaction is necessary for osteogenesis, but the latest issue argued inflammation is unavoidable in the process of bone trauma, and physiological inflammatory reaction is essential to achieve bone formation. Tissue-engineered bone graft is not only associated with osteoblast-related cells; the inflammatory reaction is the initial physiological process, mainly with neutrophil infiltration, which secretes MCP-1, IL-8, and other chemokines and further promotes dendritic cells, lymphocytes, and mononuclear macrophages to move in. The activation pathways of macrophages have a direct effect on the outcome of the inflammatory reaction and the healing, which are divided into the classical approach (M1) and the alternative approach (M2). The M1 pathway secretes IL-1 beta, IL-6, TNF-α, and other pro-inflammatory factors, while the M2 pathway secretes arginase, IL-1Ra, IL-4, and other anti-inflammatory cytokines, also with bone-healing-related growth factors, which promote homing of bone mesenchymal stem cells (bMSCs).

Keywords: tissue-engineered bone, osteogenesis, inflammatory factors, immune cells, osteoimmunology

1. Introduction

The human bone tissue has a certain self-healing ability, but for large bone defects, which measures up to more than 6–8 mm cannot self-repair. Currently, the main method for the treatment of large bone defects includes autograft and allograft, distraction osteogenesis, synthetic or natural material implanting, and bone tissue engineering [1–3]. Each method has its own advantages and disadvantages. At present, the most commonly used autograft or allogeneic bone graft has good osteogenesis, but autologous bone transplantation can cause greater trauma to the donor site, while allograft bone will inevitably have an immune response or a potential biological safety risk [4–7]. Although distraction osteogenesis can avoid the trauma of the donor area, the course of treatment is too long [8]. Synthetic or natural materials are widely distributed, but their biocompatibility is low and cannot participate in normal physiological metabolism [9, 10].

Bone tissue engineering is a new combination of life science and material science to establish human bone tissue reconstruction and recovery; its basic elements include seed cells, scaffold materials, growth factors, and biomechanics microenvironment. When amplification of stem cells cultured in vitro adsorbed on the

biocompatibility biological material that can be absorbed by the body transplanted to the damaged bone site, accompanied by biological scaffold being absorbed, osteogenic differentiation, and secretion of matrix and mineralization occur, the formation of new bone and reconstruction take place [11]. The advantages of bone tissue engineering include no extra supply area damage, no graft rejection, and no risk of the spread of the disease [12], but currently this technique can only be able to regenerate and repair some tiny bone defects, and large bone defects still remain to be solved, while the main challenge is without an independent blood supply system, which provides nutrients and growth factor; also metabolite is transported by very limited diffusion and osmosis [13, 14].

Seed cells need to meet a wide range of sources, non-immunogenicity, and a strong ability to differentiate [15]. Currently, research is mainly focused on embryonic stem cells, bone marrow mesenchymal stem cells, dental pulp stem cells, and induced pluripotent stem cells [16]. Biological scaffold materials provide space for cell adhesion, proliferation, and differentiation and promote tissue regeneration. According to their source, biological scaffolds can be divided into natural material scaffolds and synthetic material scaffolds. Natural material scaffolds include natural polymer materials, natural bone-derived materials, and natural coral bone-derived materials which advantages include widely distributed, good degradation performance, and good biocompatibility; however, disadvantages cover low mechanical strength, high brittleness, and no bone induction. Research shows that cells can receive enough nutrients and oxygen by diffusion with the distance from the capillary by 20–200 μm but cannot meet the metabolic needs beyond this distance. With the increase of scaffold material size, the internal cell microenvironment, nutrition, and oxygen gradient decreased [17, 18]. Besides, isothermal conditions, proper pH value, and adequate nutrition supply are also important factors restricting the repair of large bone defects by tissue-engineered bone.

Growth factors regulate cell division, matrix synthesis, and tissue differentiation by autocrine and paracrine [19]. Study confirmed that growth factor is related to osteogenesis induced by bone morphogenetic protein, transforming growth factor beta, platelet-derived growth factor, and insulin-like growth factor, while bone morphogenetic protein in bone tissue engineering has the most significant effect [20]; an experiment proved that its application combined with vascular endothelial growth factor shows extraordinary effect of bone healing [21].

Once the tissue engineering materials are implanted in the area, the allogenic reaction is quickly triggered, showing aseptic inflammation, and starts the tissue repair function. Tissue-engineered bone graft is not only associated with osteoblast-related cells; the inflammatory reaction is the first physiological process [22, 23]. Tissue engineering bone implantation is accompanied by an early inflammatory reaction, mainly with macrophages and neutrophil infiltration, which secretes MCP-1, IL-8, and other chemokines and further promotes dendritic cells, lymphocytes, and mononuclear macrophages to move in [24, 25]. As time goes on, neutrophils are gradually apoptotic and phagocytic by macrophages. The activation of macrophages has a direct effect on the outcome of the inflammatory reaction and the healing [26]. The activation pathway is divided into the classical approach (M1) and the alternative approach (M2). The M1 pathway secretes IL-1 beta, IL-6, TNF-α, a pro-inflammatory factor, while the M2 pathway secretes arginase, IL-1Ra, IL-4, and other anti-inflammatory cytokines, also with bone-healing-related growth factors that promote homing of bMSCs [27].

Pathological inflammatory reaction can lead to failure of fibrous wrapping and bony binding. Chemokines and cytokines produced by physiological inflammatory

reaction, such as MCP-1 and VEGF, can promote osteogenesis and angiogenesis of bMSCs. Traditionally, the inhibition of inflammatory reaction is an important means of osteogenesis, but now, it has a deeper understanding of inflammatory response. Avoiding inflammatory reaction cannot achieve its goal; physiological inflammatory reaction is essential to achieve bone formation [28, 29].

Inflammation is unavoidable in the process of bone trauma; it is considered that inflammatory reaction plays a crucial role in the process of bone healing. The concept of osteoimmunology is attracting more and more attention. The immune system plays an important role in the skeletal system, and studies have shown that many diseases are closely related to the bone immune system. Kayal, R A suggests that periodontitis is closely related to bone immunity; in the fight against foreign microbes, inflammatory cells and inflammatory mediators also activate the protease to induce matrix degradation, resulting in bone resorption [30]. Kamiya, N found the active phase of Perthes disease (LCPD), and the increased IL-6 in the synovial fluid of the synovial joint was detected [31]. Cafiero, C found that chronic kidney disease is accompanied by abnormal verification and skeletal abnormalities, which may be related to the increased expression of nuclear factor kappa B receptor-activating factor ligand (RANKL) [32]. Metzger et al. [33] have found that inflammatory bowel disease (IBD) causes the inflammatory state of the body to cause bone loss. Spinal arthropathy and progressive ossification are also diseases caused by an abnormal bone immune system. HIV-infected persons show different degrees of bone-healing disorders. This also explains to some extent that the defects of the immune system have an important impact on bone healing [34]. What are the important inflammatory factors involved in the process and the mechanism involved in osteogenesis? This article provides a framework.

2. Host initial immune response

Tissue-engineered bone graft always accompanies with implant surgery; at the same time, tissue damage is followed by a series of biochemical reactions at nanosecond level on the surface of biomaterial, which includes activation of coagulation, complement system, and immune system. Every step of these reactions significantly determines the later physiological processes; we believe that early regulation, especially initial immune regulation, is potent for tissue prognosis.

2.1 Transient protein adsorption of biomaterial implanting

When implant surgery carried on, blood coagulation factors such as fibrinogen and factor XII can be absorbed on the biomaterial surface spontaneously, which initiate coagulation cascade; also with activation of platelets, tissue factor generated by damaged tissue amplifies this process. Complement is also capable of absorbing biomaterial, especially C3 and C3b. The complement synergistically interacts with coagulation cascade, which takes advantage of adherence of platelets. Immunoregulatory function has also been found in complement, which triggers leukocyte activation and mast cell degranulation [35, 36].

Other attachment proteins include fibronectin and vitronectin in the extracellular matrix, which have the ability to activate inflammatory response and also promote osteoblast adherence. It is also worth mentioning that danger signals, which named alarmins consist of ATP, uric acid, heat shock proteins, and so on, may activate immune cells through pattern recognition receptors (PRRs), mainly with Toll-like receptors [37].

2.2 Acute inflammatory cell infiltration and pro-inflammatory factor release

Once the tissue damage occurs, polymorphonuclear leukocytes (PMNs) migrate to the damage site and initiate acute inflammation reflection. PMNs' activation is associated with integrin and PRR attached on the surface protein of biomaterial [38]. PMNs secrete a series of cytokines, which usually lead to acute inflammation; IL-6, IL-8, and CCL2 are important factors which lead to proteolytic enzymes and ROS autocrine. IL-6 is produced by osteoblasts and stimulates osteoclasts to promote bone resorption. It acts by binding to soluble IL-6 receptor (sIL-6R) present in serum and acts as an agonist to promote ubiquitin/IL-6 signal transduction. IL-6 in the body has a role in regulating immune activity, acute phase reactions, and hematopoietic activity (**Figure 1**). CCL2, also named as monocyte chemotactic protein 1 (MCP-1), has a strong chemotaxis function of recruiting monocytes which transfer into macrophages when reaching the inflammation site, also along with dendritic cells and lymphocytes. With continuous activation, PMNs undergo apoptosis within 2 days and are swallowed by macrophages. As the most diverse histiocytes, macrophages play an important role in the process of tissue remodeling [39]. It shows remarkable functional plasticity and plays a very different role in physiological and pathological environment. At present, macrophages are divided into classically activated (M1) and alternatively activated (M2) phenotypes, which are similar to Th1/Th2 subsets of helper T cells. Interferon γ (IFN-γ), lipopolysaccharide (LPS), and tumor necrosis factor-α (TNF-α) can activate the M1 phenotype, which subsequently secretes large quantities of pro-inflammatory cytokines such as IL-1β, IL-6, IL-23, TNF-α, arginine, inducible nitric oxide synthase (iNOS), and reactive oxygen species (ROS). In addition, lymphocytes, especially T cells, are proved to have an enhancement effect on macrophages [40–42].

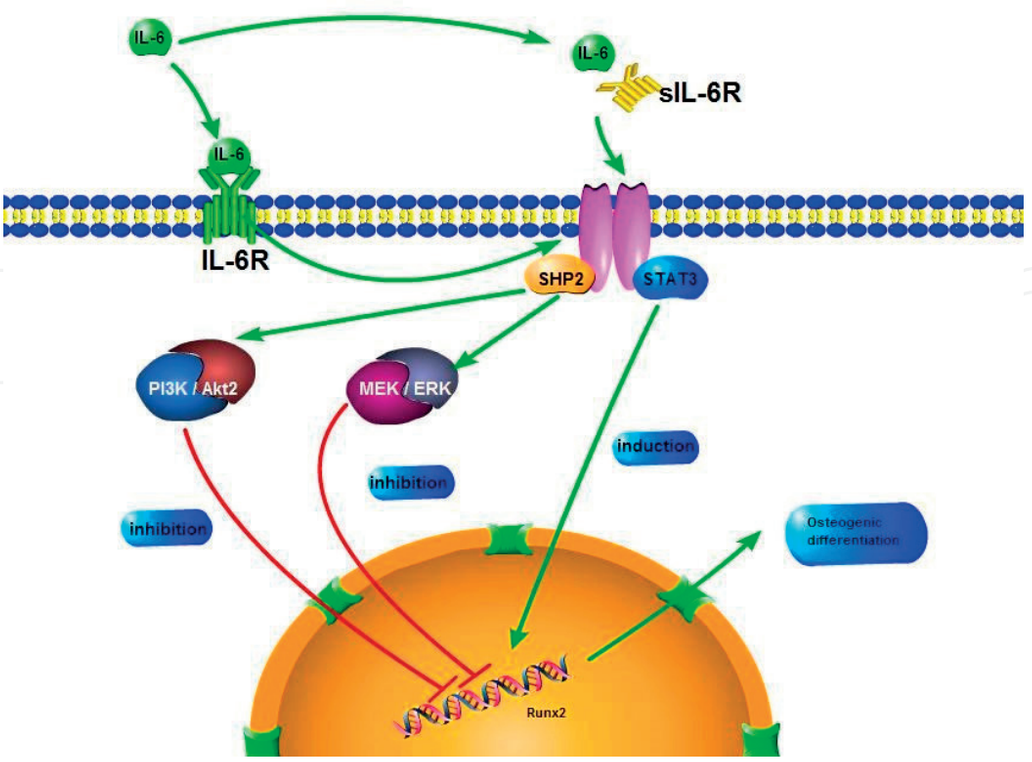

Figure 1.
IL-6 can bind to soluble IL-6 receptor (sIL-6R) present in serum and exerts its effect. IL-6 can activate two major signaling pathways, SHP2/MEK/ERK and SHP2/PI3K/Akt2, as well as JAK/STAT3 signaling pathways. IL-6 negatively regulates osteoblast differentiation through SHP2/MEK2/ERK and SHP2/PI3K/Akt2 pathways and positively regulates osteogenic differentiation through the JAK/STAT3 pathway.

2.3 BMSCs' chemotaxis

Endogenous bone marrow mesenchymal stem cells (BMSCs) have the ability to migrate spontaneously to the injured site and participate in the repair of corresponding tissues, and their directional migration depends on the interaction between chemoattractant molecules expressed locally and the corresponding receptors on the cell surface. After tissue injury, endogenous BMSCs moved out of the bone marrow, entered peripheral blood circulation and migrated to injured tissue, adhered to target vascular endothelial cells, and passed through the extracellular matrix barrier and get to the injured tissue to repair [43–46]. BMSCs are also capable to secrete a variety of growth factors that are conducive to the differentiation of hematopoietic stem cells such as IL-6, IL-11, granulocyte colony-stimulating factor (G-CSF), stem cell factor, and so on. Recent studies suggest that the main factors of chemotactic bone marrow mesenchymal stem cells are as follows: (1) Stromal cell-derived factor 1 (SDF-1) (**Figure 2**) can obviously enhance the chemotaxis function of BMSCs, the number of which is proportional to the gradient of SDF-1 concentration. Study showed that the expression of SDF-1 was significantly increased after injury of the myocardium, liver, kidney, lung, and skin to form a difference in the concentration gradient with the bone marrow, so that it may play an important role in the directional migration of bone marrow mesenchymal stem cells [47]. (2) Monocyte chemoattractant protein 1 (MCP-1)

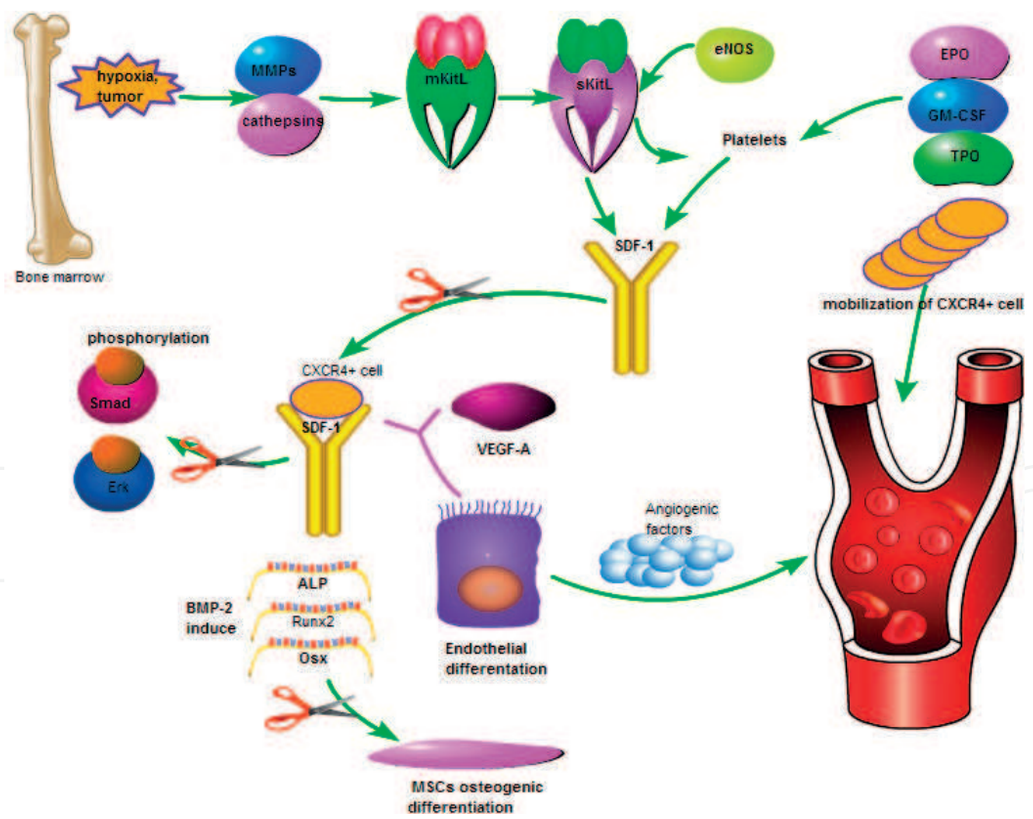

Figure 2.
Regulation of neovascularization by SDF-1. The chemokine SDF-1 is produced by hypoxic conditions, vascular injury, or tumors and is released in the circulation. SDF-1 signaling induces a complex remodeling of the BM microenvironment involving proteases, kit-ligand (KitL), and NO production, leading to mobilization of CXCR4+ angiogenic cells. Ultimately, SDF-1 expression in the neo-angiogenic niche recruits CXCR4+ cells and mediates their proper retention, differentiation, and pro-angiogenic activities in coordination with other angiogenic factors such as VEGF-A. Both PI3K/Akt and MAPK/ERK transduction pathways are involved in the enhancement of MSC migration induced via CXCR4. MSC migration was inhibited by AMD3100, LY294002, PD98059, and p38MAPK inhibitor (SB203580). Perturbing the SDF-1/CXCR4 signal axis affected the BMP2-induced osteogenic differentiation in mouse bone marrow-derived MSCs.

is a multifunctional chemokine that plays an important role in inflammatory response, injury repair, and neovascularization. Recent studies have shown that MCP-1 can promote the migration of bone marrow mesenchymal stem cells to the injured site in animal models of cerebral ischemia injury [48]. (3) Granulocyte colony-stimulating factor (G-CSF): in recent years, it has been found that G-CSF can mobilize BMSCs into blood circulation and migrate to the injury site which can be blocked by antibodies against CXCR4. Whereas BMSCs also express CXCR4, G-CSF is mobilized through a similar mechanism that remains to be further confirmed [49]. (4) Mesenchymal metalloproteinase-9 (MMP-9): Endothelial cells, fibroblasts, and inflammatory cells release interstitial metalloproteinases during inflammation or hypoxia. Recent studies have revealed that BMSCs regulate the recovery, proliferation, and differentiation of hematopoietic stem cells and endothelial stem cells through the release of soluble Kit-ligand (sKitl) mediated by mesenchymal metalloproteinase-9 to promote the migration of BMSCs by mobilizing bone marrow mesenchymal stem cells into the peripheral blood [50, 51].

3. Host anti-inflammatory phase and healing

In the late stage of acute inflammation, the polymorphonuclear granulocytes were swallowed by macrophages, and the tissues were mainly infiltrated by macrophages. Macrophages play a bidirectional role in the process of disease and tissue remodeling according to their different polarization function. Whether this action is positive or harmful depends on the transformation and balance between the polarization state of macrophage and the polarization state of M1/M2. For tissue engineering materials, the polarization of macrophages plays an important role in the function of macrophages. In view of the acute inflammatory response and infiltration of inflammatory macrophages that cannot be avoided in the early stages of implantation of bone implants, therefore, promoting the rapid and effective transformation of peri-implant inflammatory macrophages to M2 macrophages may be more helpful to promote bone healing and long-term stability of implants [42, 52, 53]. Good bone immune microenvironment can effectively promote osteogenic differentiation, while poor bone immune microenvironment can inhibit osteogenic differentiation, resulting in the formation of fibrous envelope.

3.1 Subside of acute inflammation and release of anti-inflammatory factors

Macrophages secrete a large number of bioactive factors under the certain action of tissue microenvironment. The types and secretion of these factors are closely related to the polarization and functional state of macrophages. Although there is still controversy, macrophages are divided into two major phenotypes: classically activated (M1) and alternatively activated (M2). This is very similar to the Th1/Th2 subsets of helper T cells. M1 macrophages are activated by pro-inflammatory signals such as interferon γ (IFN-γ) alone or in conjunction with lipopolysaccharide (LPS) and tumor necrosis factor-α (TNF-α) in which main surface markers were CD80 and CCR7, also known as pro-inflammatory macrophages, which secreted pro-inflammatory cytokines such as TNF-α, IL-1 β, arginine, inducible nitric oxide synthase (iNOS), and reactive oxygen species (ROS), but rarely secreted anti-inflammatory factors such as IL-10. Its main role is to kill bacteria and other pathogens and participate in the Th1-type inflammation as both the initial and effector cells. The main markers of M2 macrophages are CD163 and CD206, also known as repair macrophages. Macrophage differentiation to M2 was induced by alternative pathways such as IL-4, IL-10, and an immune complex (IC). M2 subtype can release IL-10,

IL-4, vascular endothelial growth factor (VEGF), platelet-derived growth factor BB (PDGF-BB), and transforming growth factor beta (TGF-β), which are beneficial to the formation and remodeling of new bone and extra cellular matrix (ECM) remodeling and angiogenesis [54–58]. The concentrations of pro-inflammatory factors such as IL-1 β, IL-6, and TNF-α (**Figure 3**) increased rapidly after trauma in order to initiate inflammation and bone repair in physiological environment and then gradually decreased and disappeared within 72 hours.

3.2 Osteogenesis of tissue engineering bone

Immune and vascularization are the key factors to regulate the osteogenesis of tissue engineering bone. Immune factors determine the inflammatory outcome and healing of the tissue. BMSCs have the ability to migrate spontaneously to the injured site and participate in the repair of corresponding tissues. BMSCs are a kind of low immunogenicity cells which can regulate the function of dendritic cells and T cells. It has the function of immune regulation and induction of immune tolerance and can improve and regulate the destructive inflammatory reaction [59, 60]. It is known that BMSCs secrete soluble factors through direct interaction between cells, inhibit the proliferation of T and B cells, inhibit the production of H_2O_2 secreted by neutrophils, and inhibit the cytotoxicity of T cells and NK cells. In vivo, BMSCs can differentiate into various tissue types when activated by various nutritional mechanisms, and the regeneration potential increases when exposed to the damaged environment. Cytokines such as IFN-α can regulate the homing and migration of BMSCs through the extracellular matrix [61]. Good vascularization can promote the supply of oxygen and nutrients, promote waste excretion, and accelerate the physiologic healing of tissue-engineered bone, which is another important factor in tissue engineering bone transplantation. Most of the tissues and cells in vivo are supplied with oxygen and nutrients by blood. Because of the limitation of oxygen diffusion in tissues, the oxygen supply range of capillaries can only be confined to the region of 100–200 μm [62]. Once implanted in vivo, the seed cells in the scaffold can only absorb oxygen

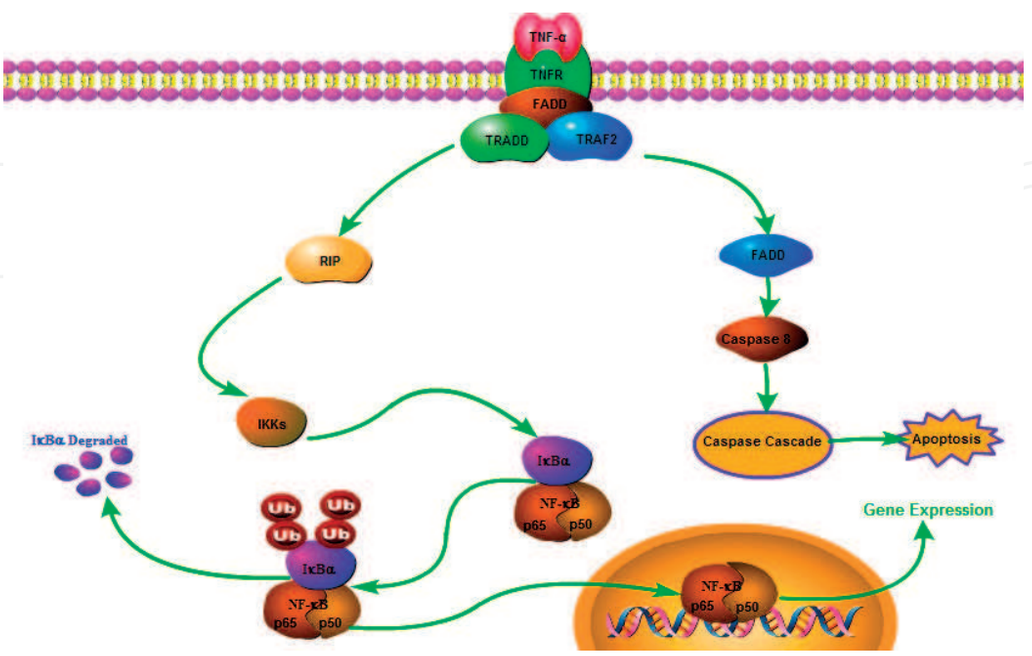

Figure 3.
TNF-α binds with TNFR1 or TNFR2 and initiates a series of signal transduction, among which FADD and NF-κB pathway determine the different destinies of the cell. The TRADD-FADD-caspase line causes apoptosis of the cell, and the TRAF2-RIP-IKKS line activates NF-κB and leads to cell survival. In fact, both routines are expressed in the cell at the same time; it is the ration of two pathways to codetermine the fate of the cell.

and nutrients through the diffusion of adjacent capillaries in the early stage. After the large tissue-engineered bone is implanted in vivo, the demand for blood supply around the scaffold is higher. The current vascularization strategies include the modification of scaffolds, the introduction of growth factors, and the combined implantation of endothelial progenitor cells. Size and roughness of materials affect immune response. Dobrovolskaia found that M1 immunoreaction was mediated when particle diameter was larger than 1 mm, and M2 immunoreaction was mediated when particle diameter was smaller than 0.5 mm [63]. Barth and other studies found that the surface roughness of materials increased, and the tendency of macrophage differentiation to M2 increased significantly, which was beneficial to promote bone regeneration [64]. Ghrebi proved that the surface roughness of biomaterials could affect the morphology of macrophages by recognizing the ERK1/ERK2 pathway activated by the adhesion protein in macrophages [65]. It is known that vascular endothelial growth factor (VEGF) [66], basic fibroblast growth factor (bFGF) [67], platelet-derived growth factor (PDGF) [67], transforming growth factor beta (TGF-β), angiopoietin l (Ang-1), and other angiogenic growth factors can promote vascularization of tissue engineering complex implanted in vivo [68]. Kim et al. encapsulated VEGF in silica nanoparticles and released them after 28 days; the study showed that this method can effectively promote angiogenesis. Endothelial progenitor cell, also known as angioblast, is involved not only in embryonic angiogenesis but also in embryonic angiogenesis [69]. Yu et al. co-cultured the endothelial progenitor cells derived from the bone marrow with osteoblasts and inoculated them on porous polycaprolactone hydroxyapatite to repair the 0.8 cm defect in the femur of rats. It was found that more capillaries and bone tissue were formed in the co-culture group than in the osteoblast group [70].

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