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Osteoimmunology: Interactions With the Immune System in Spinal Fusion

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ABSTRACT

Spinal fusion is important for the clinical success of patients undergoing surgery, and the immune system plays an increasingly recognized role. Osteoimmunology is the study of the interactions between the immune system and bone. Inflammation impacts the osteogenic, osteoconductive, and osteoinductive properties of bone grafts and substitutes and ultimately influences the success of spinal fusion. Macrophages have emerged as important cells for coordinating the immune response following spinal fusion surgery, and macrophage-derived cytokines impact each phase of bone graft healing. This review explores the cellular and molecular immune processes that regulate bone homeostasis and healing during spinal fusion.

Focus Issue Article

Keywords: spinal fusion, osteoimmunology, macrophages, bone grafts

INTRODUCTION

Spinal fusion is important for the clinical success of patients undergoing surgery, and the immune system plays an increasingly recognized role. Spinal fusion is a multifaceted and complex process that requires the coordination of a variety of cells, molecular mediators, and extracellular matrix components. The classic three stages of bone repair include the inflammatory phase, callus formation phase, and remodeling phase.[1] The immune system influences each of these phases during spinal fusion, and this review will discuss the pertinent cellular and molecular mechanisms.

THE ROLE OF INFLAMMATION

Successful bone healing is dependent on the initial acute inflammation and the innate immune response. This initial inflammatory phase of bone repair occurs in the first 2 weeks and is critical for successful spinal fusion. The Figure summarizes the impact of immune cells on bone healing following spine fusion. During the inflammation phase, neutrophils and macrophages infiltrate the fusion site and act as scavengers to clear tissue debris. Both tissue resident macrophages and inflammatory macrophages are involved;[2] inflammatory macrophages play a more prominent role immediately after bone fracture, while resident macrophages predominate in the later stages of the inflammatory phase and during the tissue healing stage.[3] Inflammatory macrophages are found within granulation tissue and are involved in soft callus to hard callus transformation, while resident macrophages persist during hard callus maturation.[4]

Macrophages involved in spinal fusion may also be activated into distinct phenotypes with proinflammatory (M1) or anti-inflammatory (M2) functions through a phenomenon called macrophage polarization.[5] M1 macrophages secrete proinflammatory cytokines including transforming growth factor beta (TGF-β), tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-6, whereas M2 macrophages secrete anti-inflammatory cytokines such as IL-4 and IL-10.[6] IL-6 and transforming growth factor (TGF)-β belong to the gp130 receptor cytokine family of receptor subunit signal transducers and are directly involved in the regulation of bone turnover, osteogenesis, and angiogenesis. M1-polarized macrophages also have the potential to differentiate into mature osteoclasts. Both M1 and M2 macrophages are attracted to the fusion site by the expression of the CXCL12 chemokine released from the damaged tissue, and this chemotaxis is enhanced by TNF-α.[7]

A key event following acute inflammation and recruitment of macrophages is the deposition of granulation tissue. Recruited mesenchymal stem cells (MSCs), osteoprogenitor cells, and fibroblasts coordinate the production of the unorganized extracellular collagen matrix. Subsequent differentiation of MSCs

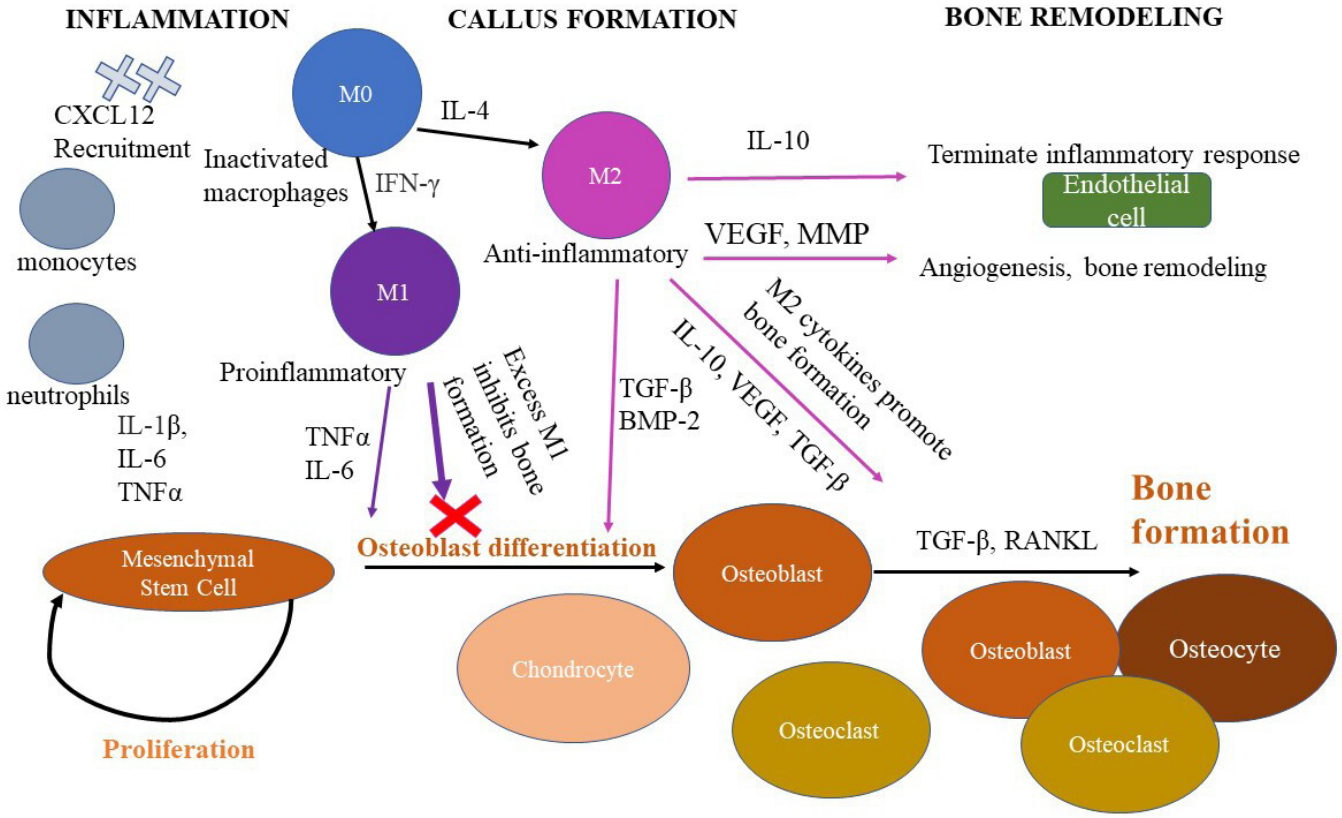

Figure. Spinal bone graft healing. Overview of immune and osteogenic cells through the 3 bone healing phases: inflammation, callus formation (repair), and remodeling. First, inflammatory cells release proinflammatory cytokines as well as anti-inflammatory cytokines. The inactivated macrophage is then polarized into a classic proinflammatory (M1) or an alternatively activated (M2) macrophage. During the callus formation, revascularization occurs with soft then hard callus formation. Finally, mature bone is formed by osteoblasts, osteocytes, and osteoclasts. The balance of M1 and M2 macrophages influences appropriate osteogenesis and bone formation. M2 macrophages promote bone formation and remodeling. Excess activation of M1s can lead to scar formation and pseudarthrosis.

toward chondrocytes produces cartilage and forms the soft callus of fusion. During the initial inflammation, macrophage-derived TNF-α (in combination with IL-6) also sensitizes osteoblast progenitors to growth factors and enhances osteoblast differentiation.[8] Osteoblasts synthesize the unmineralized osteoid of the bone matrix and then subsequently mineralize the osteoid through the secretion of alkaline phosphatase, which initiates calcium and phosphate mineralization of the matrix.

Toward the end of the inflammatory phase, the activated immune cells secrete factors to attract and stimulate mesenchymal progenitor cells, which in turn limit the inflammatory activity.[1] For instance, M2 anti-inflammatory macrophages secrete IL-10 to modulate and terminate the inflammatory response. M2 macrophages also secrete vascular endothelial growth factor and matrix metalloproteinase, which is essential for angiogenesis and tissue remodeling. This release of cytokines induces a downstream cascade that results in angiogenesis, chemotaxis, proliferation of MSC and fibroblasts, and the synthesis of the extracellular matrix.[9] The secretion of these cytokines also influences the bone healing process through the modulation of the osteogenic, osteoinductive, and osteoconductive properties of a bone graft.

While the innate immune system is well recognized for its role in acute inflammation, the adaptive immune system (through T lymphocytes) also plays an important regulatory role during bone health and disease, which are relevant for spinal fusion.[10] For instance, various T cell subtypes, including T-helper and cytotoxic T cells, are abundantly found in infiltrating bone allografts.[11] Activated T-helper 2 cells enhance the production of parathyroid hormone and maintain the anabolic activity of osteoblasts.[12] Activated T-helper 17 cells express high levels of receptor activator of nuclear factor kappa beta (RANK) ligand (RANKL) and TNF-α, which are responsible for bone destruction observed during various inflammatory conditions.[13,14] T-regulatory cells, however, are major inhibitors of bone loss through production of IL-4, IL-10, and TGF-β as well as inhibition of monocyte differentiation into osteoclasts. Abnormal T cell activity has also been linked to estrogen-deficiency-related osteoporosis through enhanced

TNF-α production, which promotes osteoclast activity.[12,13]

The other major adaptive immune cell, the B lymphocyte, does not seem to have a major regulatory role in normal bone remodeling. However, activated B cells can be potent regulators of bone resorption in disease states such as rheumatoid arthritis and malignancies such as multiple myeloma.[10]

OSTEOIMMUNOLOGY OF BONE GRAFT FUSION

Spinal fusion relies on bone graft healing; autografts, allografts, and synthetic bone substitutes are commonly used in practice. Bone graft healing is a sequential process involving inflammation, revascularization, osteogenesis, remodeling, and finally incorporation into the patient's skeletal spine to form a mechanically stable structure.[15] When a nonvascularized bone graft is implanted at the site of spinal fusion, a hematoma forms around the graft site. Necrosis around the graft incites an intense local inflammatory response,[16] which produces a fibrovascular matrix. Subsequently, host-derived angiogenesis and osteogenic precursor cells invade the graft in a process described as "creeping substitution" with incorporation of the bone graft into the host.[16] Without a robust inflammatory response, the graft may fail to revascularize, and resorption of the graft may instead occur.[16]

The three properties of the ideal bone graft material for spinal fusion include osteogenesis, osteoconduction, and osteoinduction. Osteogenesis refers to the viable progenitor stem cells that form new bone matrix and remodel bone as needed. Osteoconduction refers to the graft providing a framework or scaffold, such as bone minerals and collagen, onto which new bone can form. Osteoinduction refers to the property of the graft that contains growth factors to induce osteoblast precursors to differentiate into mature bone-forming cells. The immune system influences each of these properties during incorporation of the bone graft and will be reviewed here.

Osteogenesis

Osteogenesis is an important property for successful bone graft spinal fusion. During spinal surgery, osteogenesis can be initiated via decortication, which allows MSCs to escape from the bone marrow and into the fusion environment.[17] In the current paradigm of bone graft healing, MSCs and osteoprogenitor cells are among the first cells to enter the fusion site.[17] These cells then differentiate into osteoblasts, which deposit new bone matrix onto the transplanted bone graft or osteoconductive bone graft substitute. Once the extracellular matrix is formed, most osteoblasts either become osteocytes embedded in mineralized bone matrix or die by apoptosis.[18] Bone remodeling then occurs, resulting in a mature fusion mass. During bone remodeling, osteocytes detect the mechanical loading and regulate both bone formation (osteogenesis) primarily by osteoblasts and bone resorption (osteoclastogenesis) primarily by osteoclasts.[19]

During bone remodeling, immune cells play an important role through the regulation of osteoclastogenesis and osteogenesis. For instance, macrophages secrete IL-1 and TNF-α to inhibit collagen synthesis from osteoblasts, and TNF-α also inhibits osteoblast differentiation from precursor cells.[20,21] Conversely, macrophage-derived IL-1, IL-6, and TNF-α stimulate RANKL expression on osteoclasts to promote osteoclast activation and bone resorption.[22] RANKL is a member of TNF cytokines and stimulates the formation of osteoclasts from precursor stem cells and increases bone-resorption activity of mature osteoclasts. Furthermore, RANKL promotes macrophage recruitment, proliferation, and differentiation at the bone matrix.[23] Further research suggests that proinflammatory M1 macrophages inhibit RANKL-induced osteoclastogenesis and may represent a therapeutic osteoimmune target.[24]

T cells are also involved in the regulation of osteoclastogenesis and osteogenesis.[11] Inflammatory CD4 T cells, or TH1, secrete granulocyte-macrophage colony-stimulating factor, IL-3, and interferon (IFN)-γ, among other cytokines.[12] Granulocyte-macrophage colony-stimulating factor and IL-3 inhibit RANKL-mediated osteoclastogenesis. IFN-γ enhances the effect of macrophages on bone by promoting the activation and differentiation of macrophages.[6,25] Another type of T-helper cell, the TH2 cell, secretes IL-4 and IL-13, which inhibit osteoclast activity and bone resorption[6,26] and simultaneously recruit osteoblasts to the fusion site.[27]

Osteoconduction

Osteoconduction is another necessary property for successful spinal fusion. Bone autografts and allografts have long been used to provide structural support as well as the osteoconductive properties needed for bone healing. Autografts were the initial gold standard for spinal fusion because, in addition to their osteoconductive properties, autografts contain osteoinductive and osteogenic properties. Autologous bone is the most

efficient material and is usually harvested from the iliac crest, but this has disadvantages, including donor-site morbidity and inadequate supply. In contrast, allografts are derived from cadaveric bone and also have osteoconductive properties but have reduced osteogenic potential due to the necessary processing and sterilization prior to being implanted into a patient.

Allografts are processed through various methods to decrease graft immunogenicity and permit extended storage and distribution. Early research on bone allograft transplants recognized the potential for an immunological reaction in both patients and in animal models, which led to the development of processing techniques to reduce immunogenicity.[28,29] These processing techniques include cleaning processes that remove cellular material, irradiation, and other preservation processes such as freezing and freeze-drying (lyophilization).[30,31] Currently, allograft spinal fusion surgeries are performed without regard to human leukocyte antigen (HLA) matching because tissue matching of bone between donors and recipients is considered unnecessary.[30,32] Although bone allografts are generally considered nonimmunogenic, there is evidence that despite processing techniques, HLA-mismatching may still stimulate the immune response in humans.[33] In theory, an alloimmune bone response from the patient may contribute to suboptimal clinical events—such as rejection, delayed graft incorporation, osteolysis, infection, and fracture—but this has not been demonstrated consistently in clinical literature. One prospective study examined patients receiving cortex-replacing structural bone allografts to determine the rate of donor-specific HLA antibody sensitization and to investigate the potential effect of such HLA alloantibody sensitization on allograft incorporation. Donor-specific HLA sensitization, measured by the detection of donor-specific antibodies, occurred in 57% of patients but had no demonstrable effect on graft incorporation or union.[32] Additionally, several animal studies have found that major histocompatibility complex (MHC)–matched allografts incorporate better than MHC-mismatched allografts.[34]

The immune response promoted by the allogeneic graft appears to be chronic and continues over time, with persistence of inflammatory cytokines such as TNF-α and IFN-γ, which are involved in bone resorption, poor incorporation, and fractures.[35] Studies at the cellular level have shown that macrophages, dendritic cells, and T cells play an important role in bone graft tolerance or rejection. Dendritic cells are bone marrow-derived antigen presenting cells, which, when mature, can present an antigen via its MHC II molecules and stimulate host T cells to initiate the rejection process.[36] Both CD4 T-helper cells and CD8 T-killer cells are the major cells in the pathway of bone allograft rejection, as the cytotoxic CD8 T cells recognize antigen in association with the MHC class I molecule. The role of B cells in allograft rejection is presenting antigens to T cells, and mature and activated B cells are responsible for the production of antibodies. However, despite the fact that frozen bone allograft induces both cell-mediated and antibody-mediated cytotoxicity, the humoral response seems to have only a small effect on the graft[28] compared with cytotoxic processes.

Osteoinduction

The third property of bone graft healing is osteoinduction. A defining feature of osteoinduction is the ability to induce heterotopic bone formation when implanted in nonosseous tissue.[37] Bone growth is induced by the upregulation of osteogenic signaling pathways, such as bone morphogenic protein (BMP), and by recruitment and differentiation of osteogenic stem cells. The relationship between inflammation and bone formation along the spine has been studied in several inflammatory disease states (including ankylosing spondylitis), which provides insight into osteoimmune interactions involved with osteoinduction. In ankylosing spondylitis, there is characteristic ectopic formation of new bone and fusion of spinal segments along the axial skeleton.[38,39] Molecular investigations have revealed that inflammation-dependent expression of the osteoinductive Wnt protein is a key mediator of inflammation-induced ectopic new bone formation.[40]

The osteoinductive potential of demineralized bone matrices, cellular allograft bone matrices, and synthetic biomaterials can be assessed in vitro by measuring the osteogenic differentiation of undifferentiated MSCs or osteoprogenitor stem cells that come in contact with the material. Biomaterials with intrinsic osteoinductivity are an alternative to autograft and allograft, and a wide range of polymers, metals, composites, and ceramics have been designed for improved osteoinductive properties. The chemical and physical properties of a biomaterial influence its osteoinduction potential as well as immune response. One study found that titanium implants with high surface roughness increased M1 polarization of macrophages, which promoted osteoblast differentiation.[41] Another series of studies on microporous biphasic calcium phosphate ceramic implants found that smaller particle size (less than 20 μM) increased recruitment of macrophages, secretion

of macrophage-derived IL-6, and increased differentiation of osteoblastic cells.[42,43]

Recombinant human BMP-2 is a powerful osteoinductive agent used in spinal fusion,[44] but it can also induce an inflammatory response at supraphysiologic doses that result in clinically significant adverse events.[45,46] For instance, following lumbar spine fusion surgery with BMP-2, higher rates of radiculopathies were reported;[47] following anterior cervical spine surgery, there were increased rates of diffuse prevertebral soft tissue swelling in the neck.[48] On postoperative imaging, these effects were associated with the presence of soft tissue inflammation at the surgical site.[49] The molecular basis for the increased swelling from BMP-2 has been investigated, and an excessive inflammatory response is implicated. This BMP-2– induced inflammation has been proposed to be mediated through the nuclear factor-kB (NF-kB) pathway,[50,51] as well as the induction of inflammation through the increase in interleukins including IL-1β, IL-6, IL-10, and TNF-α.[46]

At the cellular level, BMP-2-induced inflammation has been shown to increase infiltrates of mononuclear and polymorphonuclear cells to the bone graft site.[44,52] This may occur, in part, through the chemoattractant properties of BMP-2 for lymphocytes, monocytes, and macrophages.[53] In an animal model of spinal arthrodesis, BMP-2 resulted in a systemic upregulation of IL-1β, IL-18, CCL-2, and CCL-3 levels detected in serum.[54] Interventions to reduce BMP-2-induced inflammation include the use of corticosteroids to reduce local cytokine secretion, immune cell invasion, and edema formation.[55]

OSTEOIMMUNOLOGY AND NUTRITION

The relationship between nutrition and bone health is well established, but how specific dietary nutrients impact osteoimmunology and ultimately bone healing is less well understood. The micronutrients, calcium, phosphorus, vitamin D, magnesium, and zinc, are essential for bone structure and function.[56,57] Maintaining sufficient zinc levels has demonstrated a protective effect on bone loss that is associated with the regulation of the RANKL/and osteoprotegerin (OPG) pathway with suppression of osteoclasts via downregulation of RANKL/RANK and possibly by upregulation of OPG expression, though additional study is needed to elucidate the effect of zinc supplementation.[58] Probiotics have known benefit on human health and can reduce inflammatory factors such as TNF-α and IL-1b, thereby increasing the expression of bone OPG, though further study is needed to establish direct effects of probiotics on the RANKL/RANK/OPG pathway.[59] Dietary vitamin D is of interest due to its therapeutic benefit on osteoporosis, though the active form of vitamin D3 (1 a,25-$[OH]_2$-D_3) has a dual effect of promoting osteoclastogenesis through the upregulation of RANKL/RANK/TRAF6[60] and inhibiting the proliferation of osteoclast precursors, suggesting that pharmaceuticals targeting autophagy may complement vitamin D supplementation in the treatment of osteoporosis.[61]

IMPACT OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND CORTICOSTEROIDS ON SPINAL FUSION

Clinically, the impact of immune modulatory medications such as corticosteroids and nonsteroid anti-inflammatory drugs (NSAIDs) has been studied for a possible association with pseudarthrosis following spine fusion. The molecular mechanism by which NSAIDs are proposed to interfere with bone healing is through inhibitions of cyclooxygenase-2 (COX-2) by nonselective or COX-2 selective inhibitors, which blocks prostaglandin synthesis. Prostaglandins stimulate and participate in inflammatory responses, increase osteoclast activity and subsequent bone resorption, and increase osteoblast activity and new bone formation.[62] One biochemical study tested seven COX-1 and COX-2 inhibitors on the impact of MSC differentiation. This study found that osteogenic differentiation was not inhibited by any of the COX inhibitors, but chondrogenic differentiation was reduced by COX-2 specific inhibitors parecoxib and meloxicam.[63] Several animal studies demonstrated an inhibitory effect of the nonspecific NSAID ketorolac on spinal fusion, but this was at a significantly higher dose and duration than what is routinely administered in human patients.

Clinical reports in the early 2000s suggested NSAIDs increased the rate of nonunion; there was evidence for a dose-dependent NSAID impact on nonunion that was seen with a 2-week postoperative course but was not observed when NSAIDs were only used for 48 hours after surgery.[64] One study found that the NSAID diclofenac sodium showed a dose-dependent inhibitory effect toward spine fusion, especially when used during the immediate postoperative period.[65] Many of the earlier studies were retrospective, lacked large patient cohorts, or were underpowered statistically.[64] A meta-analysis of human studies published after 2005 suggests that short-term (<2 weeks) postoperative use of ketorolac, diclofenac, celecoxib, or rofecoxib has no effect

on union rates following spinal surgery.[64,66] Instead, the adverse impact of NSAIDs on spinal fusion may be specific to surgery type, dose, and duration of use, and further investigation with a prospective double-blinded randomized placebo-controlled trial may help clarify the impact of ketorolac and other NSAIDs on spinal fusion.[67]

Clinical and animal research also has found that corticosteroids inhibit bone fusion in part through the suppression of inflammation.[68] Clinically, one prospective double-blinded randomized controlled trial looked at the effect of dexamethasone on swallowing, the airway, and arthrodesis in patients undergoing cervical fusion surgery and found that patients who were administered dexamethasone had significantly delayed fusion at 6 months but similar fusion rates by 12 months.[69] Mechanistically, corticosteroids restrict macrophage infiltration to the bone fusion site with a subsequent decrease in cytokines, which impacts bone metabolism and osteoblast differentiation.[70,71] Glucocorticoids also increase T cell production of TNF-α, which promotes osteoclast activity and bone resorption;[13,14] for this reason, T cells are implicated in chronic glucocorticoid-induced osteoporosis.[12]

EMERGING TECHNOLOGIES AND OSTEOIMMUNOLOGY

A controlled immune response contributes to successful spinal fusion, and both inflammatory and regenerative events ensure bone graft integration. New cellular, genetic, and biological therapies targeting osteoimmunology to improve bone healing have been explored. Biomaterials have the potential to induce a foreign body reaction around the implant as well as cause systemic inflammation—which can delay bone graft healing—so newer biomaterials are being designed to prevent this.[72] The ideal biomaterial would enable a controlled switch from early inflammation to recruit osteogenic stem cells to later-stage anti-inflammatory immune cells (such as M2 macrophages) to promote differentiation of precursor osteogenic cells and terminal osteoid mineralization for bone regeneration. Tissue engineering of bone can provide a biodegradable scaffold with cells or proteins to promote bone formation through the modulation of the immune system to promote osteogenic or vascular pathways. In one report on scaffold immune functionalization, a group attempted to reproduce the sequential release of polarizing macrophage cytokines such as IFN-γ, which promotes the initial activation of M1-macrophage during the first 24 hours, followed by the release of IL-4 and other cytokines that promote the alternative differentiation into M2 macrophage factors.[73] Macrophages can also be targeted to act as promoters of scaffold vascularization to promote formation of endothelial cells and M1-induced sprouting vessel anastomosis.[74] Other opportunities to engineer scaffolds for bone regeneration include using local delivery of synthetic peptides to modulate the immune response. For instance, one group engineered a scaffold that agonizes the signaling of sphyngosine-1 phosphate (S1P), a lipid which promotes the polarization of macrophages into an M2 phenotype, which resulted in enhanced osteogenesis, angiogenesis, and new bone formation in mice.[75] Another line of research reported improved spinal fusion using biphasic calcium phosphate bone grafts compared with autografts and attributed the improvement of bone formation to the enhanced efficacy of calcium phosphates with submicron topography in the upregulation of anti-inflammatory M2.[76] Overall, the functionalization of tissue scaffolds to modulate the immune response may be applied in the future to improve spinal fusion rates.

CONCLUSION

Overall, the interactions between bone and the immune system are complex. In addition to providing structural integrity for the spine, bone is the site of hematopoiesis and plays a role in immune development. Likewise, the immune system is complex and recognized to play a role in both normal and abnormal bone metabolisms. Therefore, it is important to understand the cellular and cytokine processes related to bone immunogenicity. Future research to dissect the mechanisms underlying inflammatory signaling in bone graft incorporation sites is needed as this will allow the development of safer and more successful ways of controlling the outcome of spinal fusion. Overall, modulating osteoimmune interactions is a promising strategy for bone regeneration as this could potentially improve the quality of spinal fusion.

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