Retracted: The Role of Immune System Cells in Fracture Healing: Review of the Literature and Current Concepts

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This article has been retracted.

Retraction date: June 07, 2021. Cite this retraction as Flevas D A, Papageorgiou M G, Drakopoulos P, et al. (June 07, 2021) Retraction: The Role of Immune System Cells in Fracture Healing: Review of the Literature and Current Concepts. Cureus 13(6): r32. doi:10.7759/cureus.r32.

This article has been retracted at the request of the authors. After careful consideration by our editorial staff, we have decided to grant the authors' request and retract the article. The formal request for retraction has been included below:

Dear Editorial,

This is to request the retraction of our article entitled entitled "*The Role of Immune System Cells in Fracture Healing: Review of the Literature and Current Concepts*" (ID: 20210426-13866-4cva89). As we have stressed in our previous communication on April the 30th we have found some deficits in its content and in particular, significant information not mentioned, as well as significant up-to-date knowledge omitted. Some up-to-date information was not included in our manuscript, as well as we felt that it was not scientifically solid. As the topic was very specialized, significant parts could have been added, as well as several aspects were not presented in a good scientific way. In several parts of the manuscript, the language was not correctly stated and we felt that it was not presented in the highest possible level. In its current form, this articles does not represent the work we had in mind prior to publication.

We think that a revised work, would greatly improve the manuscript and therefore it is imperative to be able to expand it and improve its presentation. This revised work would update the current paper in order for the publication to be at the highest possible level. It is our belief that a new version of the paper will be for the benefit both for us and the scientific community.

For these reasons, we would like to request the retraction of the aforementioned publication, as we will attempt to rewrite it and present it in a more scientifically solid way.

I remain at your disposal for anything further.

Best Regards,

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Abstract

Fracture healing is the most common regeneration form in clinical practice. Bone as a tissue has the unique ability to heal itself without forming a scar. After the fracture, a chain of healing reactions is activated, both at the cellular and tissue level, that lead to full bridging of the gap between the two bony ends of the fracture. There are many immune cells that take part in this healing process and they play a significant role.

There are three sequential phases to the process of fracture healing that remain independent. It has been revealed that the immune cells take part not only in the inflammation phase but also in the repair phase, where some of these cells act as intermediates to the transformation of soft callus to hard callus. In conclusion, immune cells serve as initial responders at the site of injury, restore vasculature, and initiate cascades of signals to recruit cells to carry out the repair processes. Thus the immune system can be

considered a promising therapeutic target for bone fracture healing.

Categories: Allergy/Immunology, Orthopedics

Keywords: fracture healing, immune system, bone fracture, bone healing, immune cells

Introduction And Background

Bone is a tissue that has a unique ability to heal itself without forming a scar [1]. However, it has been reported that 5%-10% of fractures result in non-union every year, a rate that increases in patients with certain comorbidities and with advanced age [1-3]. It is known that there is a need for regulation of bone homeostasis and this regulation is performed not only by the musculoskeletal system but by other biological systems as well [4]. There are various regulatory molecules that both the skeletal and immune systems share, such as cytokines and signaling molecules, and due to this the immune system is in close relation with the bone system [4,5]. This interaction between bone cells and constituents of the immune system involved in bone repair remains of great scientific interest to researchers and clinicians [6], as it is known that bone fracture healing is under the control of the immune system [4].

There are three sequential phases that comprise the process of fracture healing which however remain independent. These phases are inflammation, repair and remodeling. A local inflammatory response initiates the fracture healing process that leads to the remodeling and repair phases [7].

When a bone fracture occurs, a hematoma is formed because of the ruptured vessels that surround the bone and those in the bone marrow. Various immune cells, including neutrophils, macrophages and lymphocytes, infiltrate this hematoma [8]. These cells are believed to be activated by molecules derived by the injured tissues, and after activation these cells release pro-inflammatory cytokines at the injury site to elicit acute inflammation [4]. After the inflammation phase, mesenchymal progenitor cells gather around the bone lesion and turn into chondrocytes through the process of differentiation. These cells synthesize a cartilage matrix that forms a soft callus in order to bridge bone fragments and afterward this soft callus is replaced by hard callus consisting of bone tissue. This hard callus undergoes further remodeling by osteoclasts and osteoblasts in order to restore the original shape and function of the bone without any scar formation [4,8-10].

Review

Control of fracture healing

It is known that bone fracture healing is under the control of the immune system. The immune system is considered to be crucial for this healing process because, in the sequence of the events taking place, inflammation precedes bone regeneration. Furthermore, it has been reported that bone healing is delayed in patients who receive immunosuppressive agents. Additionally, the incidence of nonunion has been noticed to be more frequent in HIV patients [11-14].

Fracture healing consists of anabolic and catabolic phases during which both innate and adaptive immune functions are essential [8]. During the initial inflammation stage after injury, some specific cell-mediated immune functions remove necrotic tissues, promote angiogenesis and initiate repair [15-17]. While the inflammation response lasts for a short time, the effects of the immune cells extend beyond the early stages of fracture [1]. These immune cells are believed to be activated by molecules derived from the damaged tissues and then these cells release pro-inflammatory cytokines at the injury site to elicit acute inflammation [4]. The necessary first steps during the inflammation phase include clot formation, tissue granulation, and cell recruitment, which depend on the coordination of various immune cells. Hematopoietic cells seem to direct mesenchymal cell differentiation and activity throughout the different phases of this procedure [1].

An interesting fact is that fracture leads to suppression of the immune system, a situation that is achieved through a local increase in the number of induced T regulatory (iTREG) cells that suppress active adaptive immune responses within the fracture callus [18,19]. There are data that suggest that mesenchymal stem cells impart immune tolerance during the early stages of endochondral bone formation and provide protection to the developing tissues by suppressing alloproliferation of T cells during stem-cell recruitment and cartilage formation [8,19]. However, if inflammation insists or remains unresolved, like when there is a bacterial infection at the fracture site, failure to heal is possible [20].

Immune cells: function and origin

Platelets

Platelets have been shown to have a role in fracture healing although their primary function is related to blood clotting [21]. Platelets are non-nucleated cells of the myeloid lineage, which arrive at the affected site and are activated by the thrombin released in response to the injured vessels. Activated platelets participate

in the creation of the fibrin thrombus, which acts as a scaffold for cellular engraftment. At the same time, platelets secrete inflammatory cytokines (interleukin [IL]-1, IL-6, tumor necrosis factor [TNF]- α) and growth factors (platelet-derived growth factor [PDGF], TGF- β), in order to recruit other immune cells (neutrophils and monocytes) and mesenchymal progenitor cells [22-24].

Macrophages

Macrophages are myeloid lineage cells that are differentiated from monocytes. They consist of various cell subsets with different functions and are responsible for arranging inflammation and tissue regeneration after injury [25,26]. Macrophages are some of the earliest cells that emerge in the hematoma and remain present throughout the healing process [8,27,28]. They play an integral part in bone homeostasis and in bone fracture repair as they seem to act as niche cells to osteoblasts and to osteoclasts, taking part in the crosstalk and communication to maintain the balance in bone remodeling [1,29]. Macrophages can be divided into two subgroups: M1 macrophages (classically activated) and M2 macrophages (alternatively activated). A 2015 study suggests that M1 macrophages preferentially infiltrate into the bone fracture site in the acute phase, while M2 macrophages increase in number in the subacute phase [30]. Both M1 and M2 macrophages play significant roles in the healing process by regulating the early and later phases. This is indicated by the fact that temporal depletion of macrophages in either the acute or subacute phase leads to significant delay of bone healing [31]. Additionally, depletion of macrophages decreases the number of mesenchymal progenitor cells and inhibits the ability of these cells to differentiate to osteoblasts [29,32].

Although the importance of macrophages in tissue homeostasis has been confirmed in other tissues as well and it is known that macrophages produce many types of effector molecules, it still remains unclear which molecules mediate such effects and the importance of macrophages in fracture healing is still being investigated [1,4].

Neutrophils

The role of neutrophils in fracture healing remains unclear and involves many aspects of tissue repair. Neutrophils consist of phagocytic cells of the myeloid lineage and are recruited by IL-1 and TNF- α which are secreted by platelets [33,34]. Neutrophils contribute to both the early and later stages of the inflammatory phase. In the early stages of the inflammatory phase they contribute to the fibrin thrombus by depositing a fibronectin matrix, while during the later stages of the inflammatory phase, neutrophils take part in removing cellular and tissue debris and are implicated at the thrombus removal [17,35-37]. The neutrophils arrive first to the fracture site and they have an anti-septic effect and also clear the damaged cells and debris [17,36-37]. The most significant role of the neutrophils seems to be the secretion of cytokines (IL-1, IL-6, IL-10, TNF- α , monocyte chemoattractant protein-1 [MCP-1], CXC ligand-1 α [CXCL-1 α], macrophage inflammatory protein-1 [MIP-1]) in order to attract monocytes, which will differentiate to macrophages [1].

T Lymphocytes

T lymphocytes, also known as T cells, are hematopoietic cells of the lymphoid lineage characterized by the expression of T-cell receptors (TCRs) [1,4]. Studies that used animal models showed that T lymphocytes, and B lymphocytes as well, are recruited at the fracture site after three days of injury and then their numbers are reduced when the cartilaginous formation begins [38,39]. It has been noticed that depletion of T cells leads to diminished bone health and subsequently to decreased fracture healing [38,40]. Studies with mice have shown that subjects with a lack of T and B lymphocytes have stiffer bones that are more susceptible to fracture [41].

It is known that bone fracture healing is regulated by T lymphocytes, which are present at the injury site both at the early and late phases of the healing process [42]. T lymphocytes are characterized by the expression of TCRs, according to which they are classified into $\alpha\beta$ T cells or $\gamma\delta$ T cells. $\alpha\beta$ T cells go through positive and negative selection in the thymus in order to mature and express $\alpha\beta$ TCRs and co-receptors [43], generating CD4⁺ T helper (Th) cells, CD8⁺ cytotoxic T lymphocytes (CTLs), and CD4⁺ regulatory T cells (Tregs) [44]. $\gamma\delta$ T cells come from the same precursor cells as $\alpha\beta$ T cells, but they recognize not antigen-specific molecules [45]. They recognize both microbial and self components that are released due to tissue stress, contributing to the innate immune responses by cytokine production and/or cytotoxicity [46]. Also, $\gamma\delta$ T cells are classified into subsets according to the expression of TCR-V γ chains, and each subset possesses its own characteristic tissue distribution and cytokine production pattern [47].

While $\alpha\beta$ T cells exhibit pro- inflammatory and anti-inflammatory functions that are crucial for antigen-specific immune responses, $\gamma\delta$ T cells are reported to preferentially distribute to epithelial tissues in order to play a role in host defense and tissue repair in the periphery [4]. It was reported recently that $\gamma\delta$ T cells were found to contribute to bone fracture healing by robustly increasing in number after bone injury and produce IL-17A, which enhances bone formation [48].

Furthermore, it is reported that effector memory CD8⁺ T cells are enriched in the peripheral blood of patients with delayed fracture healing, while in mice injected with CD8⁺ T cells, bone healing underwent a

significant delay. These facts indicate the suppressive effect of these cells on bone repair [49]. However, despite the fact that CD8+ T cells can produce TNF- α and interferon (IFN)- γ , which suppress the mineralization of bone marrow mesenchymal stem cells (MSCs), the precise mechanisms remain unclear [4].

B Lymphocytes

B lymphocytes, also known B cells, are hematopoietic cells of the lymphoid lineage, just like T lymphocytes, and play a central role in humoral immunity [1,4]. Depletion of B cells leads to diminished bone health and decreased fracture healing, same as the lack of T cells [38,40]. It is known that B lymphocytes produce cytokines, which exert many functions [50]. The B lymphocytes that encounter their cognate antigens begin to proliferate and differentiate into plasma cells that produce antibodies to protect the body. Some B lymphocytes differentiate into memory B cells that are prepared to act in re-infection. Additionally, a number of B cell subsets with different functions have now been reported [4].

B lymphocytes and T lymphocytes seem to play a cell-signaling role near the end of the inflammatory phase and again during the mineralization phase [42]. During later stages of the inflammatory phase, while T cells produce receptor activator of nuclear factor kappa-B ligand (RANKL) in order to recruit, differentiate, and activate osteoclasts, B cells are involved in suppression of the pro-inflammatory signals IFN- γ , TNF- α , and IL-2 [51]. At the same time, B cells also produce osteoprotegerin (OPG), regulating in that way osteoclastic differentiation and activity [5,52,53].

Initially, B lymphocytes were thought to not have a significant role in bone fracture healing because a study reported that mice with deficiency in B cells, due to a lack of the μ chains of B-cell receptors, had no impairment in bone healing [54]. However, it is known that during fracture healing B lymphocytes increase at the injury site and in the peripheral blood and it has been shown that low production of IL-10 by B cells is associated with delayed fracture healing [40,42,51,55]. There are also functions of other B-cell subsets in bone regeneration that still remain largely unknown and thus further studies are required.

Natural Killer Cells

Natural killer cells (NK cells) are hematopoietic cells of the lymphoid lineage and their function in fracture healing is not fully known [1]. Their immunological function is to recognize foreign or virally infected cells and induce apoptosis or cell lysis through cytotoxic granules [56]. This function in fracture healing is possibly expressed by removing damaged cells in the injury site, while it has been shown that conditions at the fracture site inhibit NK cell-based cell lysis [57]. However, it seems more likely that NK cells play a signaling role in debridement of the injured tissue recruiting inflammatory cells and osteoclasts since it is known that they produce IFN- γ and RANKL [58]. Furthermore, they may take part in tissue deposition through recruitment of mesenchymal progenitor cells during later stages of fracture healing [59].

Pro-Inflammatory Cytokines

When bone injury occurs, one early event that takes place is the interruption of blood supply and platelet aggregation with the release of platelet-derived pro-inflammatory cytokines, IL-6, IL-1, and TNF- α [6]. This phase also includes the formation of haematoma, which traps inflammatory cells that further produce pro-inflammatory inflammatory cytokines and growth factors. The formation of this haematoma is crucial and its removal causes defective bone healing [6,60,61].

Interleukin 6

IL-6 is produced by a variety of cell types and has receptors that are expressed on a variety of cell types. Thus IL-6 exerts multiple effects and it is well-established that IL-6 enhances osteoclastogenesis [62-64]. IL-6 is induced within a day after the fracture and it is shown that it promotes the osteoblastogenesis of mesenchymal cells, including cells in the bone repair site. However, this healing impairment is limited to the early phase possibly due to the fact that other factors of the immune system compensate for IL-6 deficiency [65-67]. Additionally, it seems that the effects of IL-6 on bone metabolism are dependent on the conditions in which IL-6 is produced. This possibly occurs because numerous cell types express IL-6 receptors and the IL-6-responding cells are different. However, the source of IL-6 in bone fracture healing remains to be identified in future studies [4].

Tumor Necrosis Factor- α

TNF- α is mainly produced by activated macrophages and is able to bind to TNFR1 (p55, encoded by the *Tnfrsf1a* gene) and TNFR2 (p75, encoded by the *Tnfrsf1b* gene) in order to transduce its signals [4,68]. TNF- α is thought to be detrimental to bone because in studies conducted with mice, those carrying a human TNF- α transgene developed chronic inflammatory polyarthritis [69,70]. Furthermore, it is reported that TNF- α induced an inhibitor of Wnt signaling in synovial fibroblasts so as to suppress bone formation in the arthritic joint, while in the context of bone fracture healing, TNF- α was shown to promote bone regeneration [71,72].

There are in vitro experiments in which mesenchymal cells treated with TNF- α showed either a promoting or suppressing effect on bone formation. This condition may be due to the differential expression patterns of TNF- α receptors and signaling molecules [4].

Interleukin 17A

Initially, IL-17A was found to be produced by Th17 cells and subsequently, it has been shown that is also produced by CD8⁺ T cells, $\gamma\delta$ T cells, invariant natural killer T cells, NK cells, lymphoid tissue inducer (LTi) cells, B cells, and mesenchymal cells [73-77]. IL-17A is known to be related to inflammatory bone loss in erosive arthritis and provides protection against bacterial and fungal infections by stimulating the production of antimicrobial peptides and recruiting neutrophils [4]. Furthermore there are studies that suggest that IL-17A promotes bone formation and that an anti-IL-17A antibody is effective for arthritis with abnormal bone formation [78,79].

IL-17A is induced in the early phase of bone fracture healing, with its major source being the $\gamma\delta$ T cells [19,48], and it promotes the proliferation of mesenchymal cells in the injured tissue and their subsequent differentiation into osteoblasts [48]. Taking into account the fact that IL-17A is highly induced in the period immediately after fracture, it is possible that this cytokine positively regulates the early phase of osteoblastogenesis [4].

Osteoclasts

Osteoclasts are not traditionally thought of as immune cells. However, they are able to act as innate immune cells within bone as inflammatory signals lead to their differentiation and activation [1,80]. Osteoclasts are multinucleated cells of the myeloid lineage. They differentiate directly from monocytes, but they can also arise from macrophages [81]. Their primary role is that of a 'bone phagocyte' and they are specialized cells since they are the only cells with the ability to resorb bone matrix [82].

There is an immune cell signaling that regulates osteoclast activation. During fracture healing, monocytes are recruited to the injury site and differentiate into osteoclasts that reside on mineralized bone surfaces. Osteoclasts are primarily activated by RANKL, which binds to the osteoclast cell surface receptor RANK [1]. Osteoblasts seem to be the primary source of RANKL during homeostasis and fracture healing. However, NK cells and activated T cells are also able to produce RANKL during fracture healing. On the other hand, OPG is a decoy receptor that binds to RANK and inhibits RANKL binding and that way prevents osteoclast activation. OPG is secreted by osteoblasts during homeostasis and fracture healing and by B cells during fracture healing [1].

Conclusions

There are many immune cells that take part in fracture healing by playing a critical role in the whole process. Bone fracture healing consists of three phases: inflammation, repair and remodeling. The process starts with the crucial phase of inflammation, in which both innate and adaptive immune cells and also cells of macrophage-osteoclast lineage help with the removal of bone debris, antisepsis and preparation of MSCs for the next repair phase. MSCs are identified as cells with adherence capacity, which express surface molecules CD90, CD73, CD105, but not hematopoietic lineage markers, and are able to differentiate into bone, fat and cartilage cells.

The roles of immune cells involved in regulation of the healing process have been revealed not only in the inflammation phase but also during the repair phase, where certain immune cells and mediators play an important role to convert soft callus into hard callus and form new blood vessels. Furthermore, the bone remodeling phase is mediated through interaction between osteoclasts and osteoblasts under the influence of MSCs, macrophages and probably Th17 lymphocytes.

In conclusion, immune cells serve as the initial responders at the site of injury, restore vasculature, and initiate cascades of signals to recruit cells to carry out the repair processes. Thus the immune system can be considered a promising therapeutic target for bone fracture healing. However, there are some issues that remain to be clarified and a more thorough understanding of the regulation of bone metabolism by the immune system is required in order to better appreciate the biological significance of immune cells in bone regeneration.

Additional Information

Disclosures

Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no

other relationships or activities that could appear to have influenced the submitted work.

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