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Clinical relevance of altered bone immunopathology pathways around the elbow

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Abstract. – Normal healing of fractures is a complex process that relies heavily on a cascade of consecutive activations of immune cells and mediators. This mechanism somewhat overlaps with all processes related to bone metabolism, from the absence of unions to heterotopic ossifications and osteoporosis. We aimed to review and describe this intricate process of bone metabolism with particular focus on abnormal function and to exemplify it with a series of clinical cases which could justify their practical importance. The elbow has great potential for fracture healing but it is very sensitive to prolonged immobilization which can easily lead to intra-articular adherences and stiffness. In addition, the interosseus membrane facilitates communication between the regenerative environments when both radius and ulna are fractured. Such extensive injuries, around the proximal forearm, can lead to heterotopic ossifications and synostosis, which decrease sagittal range of motion through impingement and even block rotational movement through bone bridges. Increased knowledge and awareness of the biological mechanism of fracture healing, will have great improvement in the pharmacological adjuvant treatment of elbow injuries.

Key Words:
Radio-ulnar synostosis, Immunopathology, Fracture healing, Hematoma calcification, Heterotopic ossification.

Introduction

Activations of immune cells and mediators is a physiological cascade normally involved into a bone fracture healing process, which somewhat overlaps with all processes involving bone metabolism, including lack of unions, heterotopic ossifications and osteoporosis. Clinical observations regarding the healing behavior of the injured elbow were firstly collected founding that the elbow has great potential for fracture healing but is very sensitive to prolonged immobilization which can easily lead to intra-articular adherences and stiffness. In addition, when both radius and ulna are fractured, the inter-osseus membrane facilitates communication between the regenerative environments. Such extensive injuries around the proximal forearm can lead to heterotopic ossifications and synostosis which decreases sagittal range of motion through impingement and even block the rotational movement through bone bridges. The inflammatory phase is one of the initiating factors for bone healing. The exact role of the various cytokines involved in bone healing on osteoblast biology is not entirely clarified[1]. The understanding of the molecular and cellular mechanism of fracture healing can facilitate the fracture management and the treatment of impaired bone healing[2]. System wide inflammatory conditions also modulate the primary processes of fracture management which could explain the shock induction in polytraumatic patients, as well as increased ossifications associated with head injuries. We aimed to review and describe this intricate process of bone metabolism, with particular focus on abnormal function and exemplifying it with a series of clinical cases that will justify their practical importance.

Methods

Scope of the present review is to analyze all the recent literature focused on the immunopathological pathways around the elbow joint in order to identify relevant topics which could be useful to improve clinical practice. In addition,

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we searched on the electronic database of our clinic, over a period of 5 years, in order to obtain a broad view of the surgically treated elbow fractures/dislocations. At the end we refined the results in order to discard multiple or inappropriate coding. Virtually, all simple radial head fractures and elbow dislocations were treated as outpatients and, thus, not included in our results. We then pursued to identify the surgical treatment for any complication of the elbow joint regardless its etiology and providing evidence for relevant cases.

Results

Three directions of clinically relevant immune-pathological researches were identified: "*fracture healing* and *non-union*", "*induced membranes technique*" and "*heterotopic ossifications*". Out of 106 admissions for traumatic injuries about the elbow, the majority were olecranon fractures, followed by fractures of the distal humerus. Virtually all osteo-synthesis for the olecranon were performed using K wires and figure eight cerclage, with a trend towards bicortical fixation. This construct was also the most frequent to require removal and had favorable outcomes. The distal humerus fractures, on the other hand, often led to ROM limitations and ossifications which persisted even after implant removal. A total of 14 cases were surgically treated for important residual functional limitations: 6 distal humerus fractures, 4 unstable dislocations (terrible triad), one distal humerus non union, one radioulnar proximal synostosis, one bad connected proximal ulna fracture and one extended tumoral resection of the proximal ulna.

Fracture Healing and Lack of Union

Whenever a fracture occurs, bone and surrounding soft tissues are ruptured. The immediate consequence is the release of inflammatory mediators and the formation of an hematoma. This is deemed the acute inflammatory phase; it peaks within 24 hours and develops under hypoxic conditions. The tumor necrosis factor-α (TNF-α) and interleukins 1 and 6 (IL-1, IL-6) are the major regulators[3]. Then, the callus fills with cartilage formed from specialized mature mesenchymal stem cells recruited by stromal cell-derived factor-1 and G-protein-coupled receptor CXCR-4[4]. Vascular endothelial growth factor (VEGF)-dependent pathway is the responsible for revascularization and neo-angiogenesis at the fracture site. The cartilage then calcifies and is replaced with woven bone which confers rigidity[3]. In an animal model, a study by Toben et al[5] compared the healing process of a fracture between normal and imunodeficient hosts. Recombination of activated gene 1 deprived (RAG1-/-) organisms showed more bone and less cartilage with an accelerated endochondral ossification. In addition, they had less lymphocytes and reduced expression of inflammatory cytokines apart from IL-10. Nam et al[6] performed a similar research using immunodeficient (recombination activating gene 2) mice as a model of impaired injury repair. IL-17F was determined to be an important contributor for the cellular response in osteogenesis and supposed to be produced by Th17 subset of T-lymphocytes. General administration of prostaglandin (Pg) E receptor 4 ligands, such as prostaglandin E2, appears to support fracture healing. In a study by Tanaka et al[7], the total volume of cortical bone, as well as the mineral content, increased proportionally with the Pg dose by accelerating the local turnover. A skeletogenesis regulator, the beta-catenin pathway, activates T cell factor dependent transcription and positively regulates osteoblasts. Chen et al[8] demonstrated that, in early stages of fracture repair, beta-catenin differentiates pluripotent mesenchymal cells to either osteoblasts or chondrocytes. Afterwards, beta-catenin continues to exert a positive regulation on osteoblasts. Sclerostin is a glycoprotein secreted by osteocytes which inhibits osteoblastogenesis via Wnt signaling. Furthermore, sclerostin neutralizes antibodies leading to increased bone mineralization in animal models of osteoporosis. Systemic administration of Scl-Ab III also results in an increased mineral density and histological bone deposition in a non-critical size defect as early as the first week. These findings might provide its potential use in complicated fractures and non-unions[9,10]. Osteoclasts cross presents antigens to induce transcription factor Scurfin (also known as forkhead box P3 and encoded by Foxp3) in CD8+ T-cell. In an animal model for hormonal osteoporosis, this process was showed to limite bone loss while to increase bone density[11] (Figure 1).

Induced Membranes Technique

Inducement of foreign-body granulation tissue is a promising aid treatment for large bony defects repair. Masquelet and Begue[12] found that, when a segmental bone loss is temporarily occu-

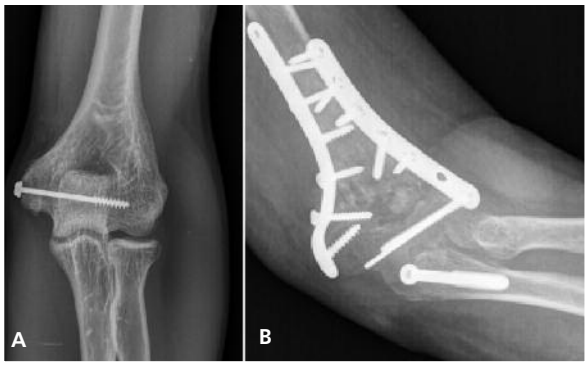

Figure 1. ***A,*** Normal bone healing of a medial epicondyle fracture; after surgical treatment, the local conditions allowed for a complete mineralization and disappearance of fracture line at five months follow-up. ***B,*** By contrast, this comminuted distal humerus fracture shows clear signs of supracondylar nonunion at three months and was operated again.

pied by a polymethylmethacrylate (PMMA) spacer, a reactive inflammatory membrane creates around it in as little as 6 weeks. In a second step, when the cement block is removed and replaced by cancellous bone, this membrane acts, somewhat, like a periost, preventing resorption and secreting growth factors. A literature review by Taylor et al[13] detailed the benefits of foreign-body induced membranes in the staged treatment of segmental bone defects. They point out that PMMA cement induces a biologic membrane that will nurture the definitive bone graft. One to two months after spacer placement the protective shell matures. This prevents graft dispersal and resorption, promotes revascularization and induces growth factors that lead to excellent clinical results being reported. The inductive potential of such membranes has been histological proved in animal models[14,15] with better results in comparison to recent artificial bioresorbable polylactide membranes that boast single step procedures[16]. Many authors[15,17] have now showed that production of growth (VEGF, TGF-β1) and osteoinductive factors (BMP-2) will peak as early as one month. This well correlated with the expression of VEGF, IL-6 and type-I collagen, as well as type-I procollagen production in aminoterminal propeptide, ionic calcium concentration increase and alkaline phosphatase increased activity when co-cultured on mesenchymal cells[15]. Such immunochemistry analysis can support and confirm a more rapid conversion to bone grafting[15,17] (Figure 2).

Heterotopic Ossification

Heterotopic ossifications around the elbow are very common post traumatic findings and a source of bony impingement which interferes with normal range of motion[18]. Inducement of heterotopic bone formation led to the identification of bone morphogenetic proteins. These are extracellular cytokines of the TGF-β (transforming growth factor) family. TGF-β supergene family stimulates new endochondral bone production by mimicking stages from the embryonic development[19].

BMPs are regulated via transmembrane type I and type II serine/threonine kinase receptors and intracellular SMAD proteins. Smad1/5/8 transcription factors get phosphorylated at the C-terminal SVs by BMP type I receptors and thus start the transcription of early BMP-responsive genes by coupling to sequences in the enhancer regions. Overactive signaling leads to heterotopic ossification[20,21].

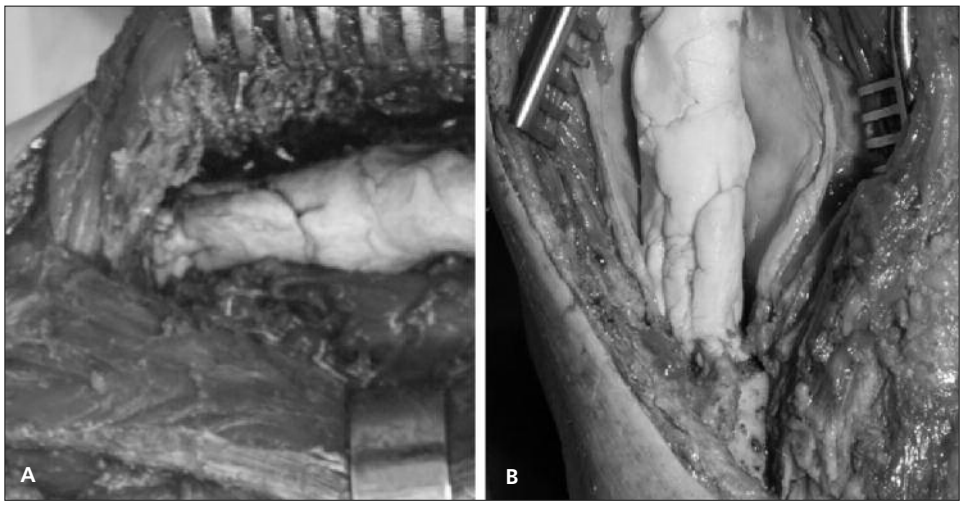

Figure 2. ***A,*** Large proximal ulna defect filled with PMMA spacer. ***B,*** The same case during secondary intervention; the cement block is seen surrounded by a clear membrane which will support the bone graft.

Recombinant human bone morphogenetic protein-2 (rhBMP-2) is used in spinal fusion to aid with consolidation. Daily practice somewhat encountered frequent and somewhat occasionally important complications associated with their use[22]. The bone morphogenetic proteins are particularly attractive in minimally invasive lumbar fusion, because here more surfaces are inappropriately prepared compared with the open procedure. By using a combination of low-dose BMP in addition to bone autograft, specific complications could be reduced while still achieving fast and reliable fusion[23] (Figure 3).

Discussion

Osteo-immunology extends to more other factors than fracture healing and bone mineralization. Rheumatoid arthritis, osteoporosis, multiple myeloma and breast/prostate cancer are also advancing through modern conditional gene targeting and transgenic technologies[24]. COX-2 inhibitors impede inflammation by down regulation of pro-inflammatory prostaglandins from arachidonic acid and therefore impair fracture healing[25]. Among the current NSAIDs in use, indomethacin was clearly proved to interfere with callus formation and fracture healing. Apart from the biological processes, mechanical fixation is required for consolidation. The current principles of internal stabilization of the fracture respect these prerogatives. The most common form of bone healing is known as secondary repair. It consists of both endochondral and intramembranous regeneration. From a macro, clinical perspective, it is important to know that it does not necessarily require anatomical reduction or rigidly stable conditions[1]. In order to minimize local disturbance and dilution of mediators and fracture hematoma, whenever possible, closed reduction and fixation is performed. This allows for an optimum cellular regeneration and mineralization of the callus and leads to favorable outcomes. Any alteration of these processes delays the fracture healing. The causes can be multifactorial. Local variables involve improper biomechanical stability, incomplete reduction of the fracture gap, infection or aggressive surgical treatment which devitalizes large portions of tissue. Systemic factors are all conditions interfering with the bodies capability to regenerate and deposit bone, from nutrient depletion (vitamins C and D, calcium and proteic intake or absorption), lifestyle conditions such as smoking and/or alcoholism, to metabolic dysfunctions of the bone cycle hormones and regulators (parathyroid, diabetes mellitus, renal insufficiency, menopause, chronic steroid use) and senile osteoporosis.

Conclusions

The elbow is a complex joint that controls the functional length of the upper limb. Just as important, the articulation between the radial head and the capitellum, as well as its relationship to the proximal ulna permits a fluent prono-supination. Over the last years an increase in complex patterns (in both coronal and sagittal planes) and very distal fractures in osteoporotic bone has been reported. The intricate articular surfaces make internal fixation difficult. In addition, increased susceptibility for stiffness limits the im-

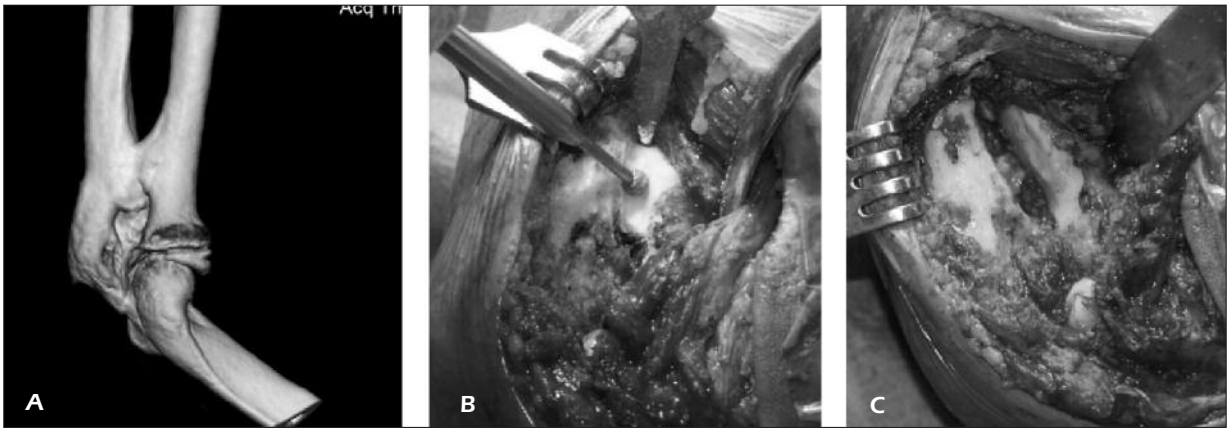

Figure 3. ***A,*** Preoperative 3D reconstructed CT volume (VRT) depicting proximal radio-ulnar synostosis and malunited olecranon fracture. ***B,*** Intraoperative aspect while burring of the osseous bridge. ***C,*** Final result which permits prono-supination.

mobilization and justifies all efforts for rapid consolidation. We, therefore, believe that increased knowledge of the biological mechanism of fracture healing will produce a great improvement in the pharmacological adjuvant treatment of elbow injuries.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Marsell R, Einhorn TA. The biology of fracture healing. Injury 2011; 42: 551-555.
2) Pape HC, Marcucio R, Humphrey C, Colnot C, Knobe M, Harvey EJ. Trauma-induced inflammation and fracture healing. J Orthop Trauma 2010; 24: 522-525.
3) Gerstenfeld LC, Cullinane DM, Barnes GL, et al. Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. J Cell Biochem 2003; 88: 873-884.
4) Kitaori T, Ito H, Schwarz EM, et al. Stromal cell-derived factor 1/CXCR4 signaling is critical for the recruitment of mesenchymal stem cells to the fracture site during skeletal repair in a mouse model. Arthritis Rheum 2009; 60: 813-823.
5) Toben D, Schroeder I, El Khassawna T, Mehta M, Hoffmann JE, Frisch JT, Schell H, Lienau J, Serra A, Radbruch A, Duda GN. Fracture healing is accelerated in the absence of the adaptive immune system. J Bone Miner Res 2011; 26: 113-124.
6) Nam D, Mau E, Wang Y, Wright D, Silkstone D, Whetstone H, Whyne C, Alman B. T-lymphocytes enable osteoblast maturation via IL-17F during the early phase of fracture. Repair PLoS One 2012; 7: e40044.
7) Tanaka M, Sakai A, Uchida S, Tanaka S, Nagashima M, Katayama T, Yamaguchi K, Nakamura T. Prostaglandin E2 receptor (EP4) selective agonist (ONO-4819.CD) accelerates bone repair of femoral cortex after drill-hole injury associated with local upregulation of bone turnover in mature rats. Bone 2004; 34: 940-948.
8) Chen Y, Whetstone HC, Lin AC, Nadesan P, Wei Q, Poon R, Alman BA. Beta-catenin signaling plays a disparate role in different phases of fracture repair: implications for therapy to improve bone healing. PLoS Med 2007; 4: e249.
9) Jawad MU, Fritton KE, Ma T, Ren PG, Goodman SB, Ke HZ, Babij P, Genovese MC. Effects of sclerostin antibody on healing of a non-critical size femoral bone defect. J Orthop Res 2013; 31: 155-163.
10) Virk MS, Alaee F, Tang H, Ominsky MS, Ke HZ, Lieberman JR. Systemic administration of sclerostin antibody enhances bone repair in a critical-sized femoral defect in a rat model. J Bone Joint Surg Am 2013; 95: 694-701.
11) Buchwald ZS, Kiesel JR, Yang C, Dipaolo R, Novack DV, Aurora R. Osteoclast-induced Foxp3+ CD8 T-cells limit bone loss in mice. Bone 2013; 56: 163-173.
12) Masquelet AC, Begue T. The concept of induced membrane for reconstruction of long bone defects. Orthop Clin North Am 2010; 41: 27-37.
13) Taylor BC, French BG, Fowler TT, Russell J, Poka A. Induced membrane technique for reconstruction to manage bone loss. J Am Acad Orthop Surg 2012; 20: 142-150.
14) Klaue K, Knothe U, Anton C, Pfluger DH, Stoddart M, Masquelet AC, Perren SM. Bone regeneration in long-bone defects: tissue compartmentalisation? In vivo study on bone defects in sheep. Injury 2009; 40(Suppl 4): S95-102.
15) Aho OM, Lehenkari P, Ristiniemi J, Lehtonen S, Risteli J, Leskelä HV. The mechanism of action of induced membranes in bone repair. J Bone Joint Surg Am 2013; 95: 597-604.
16) Meinig RP. Clinical use of resorbable polymeric membranes in the treatment of bone defects. Orthop Clin North Am 2010; 41: 39-47.
17) Pelissier P, Masquelet AC, Bareille R, Pelissier SM, Amedee J. Induced membranes secrete growth factors including vascular and osteoinductive factors and could stimulate bone regeneration. J Orthop Res 2004; 22: 73-79.
18) Foruria AM, Augustin S, Morrey BF, Sánchez-Sotelo J. Heterotopic ossification after surgery for fractures and fracture-dislocations involving the proximal aspect of the radius or ulna. J Bone Joint Surg Am 2013; 95: e66.
19) Ripamonti U, Ferretti C, Heliotis M. Soluble and insoluble signals and the induction of bone formation: molecular therapeutics recapitulating development. J Anat 2006; 209: 447-468
20) Shi S, de Gorter DJJ, Hoogaars WM, 't Hoen PAC, ten Dijke P. Overactive bone morphogenetic protein signaling in heterotopic ossification and Duchenne muscular dystrophy. Cell Mol Life Sci 2013; 70: 407-423.
21) Katagiri T, Tsukamoto S. The unique activity of bone morphogenetic proteins in bone: a critical role of the Smad signaling pathway. Biol Chem 2013; 394: 703-714.
22) Carragee EJ, Hurwitz EL, Weiner BK. A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned. Spine J 2011; 11: 471-491.
23) Mannion RJ, Nowitzke AM, Wood MJ. Promoting fusion in minimally invasive lumbar interbody stabilization with low-dose bone morphogenic protein-2--but what is the cost? Spine J 2011; 11: 527-533.
24) Danks L, Takayanagi H. Immunology and bone. J Biochem 2013; 154: 29-39.
25) Manigrasso MB, O'Connor JP. Accelerated fracture healing in mice lacking the 5-lipoxygenase gene. Acta Orthop 2010; 81: 748-755.