Mini Review

The roles of IL-1 receptor antagonist in skin wound healing

Yuko Ishida[1,*], Naofumi Mukaida[2], and Toshikazu Kondo[1]

[1]Department of Forensic Medicine, Wakayama Medical University, Wakayama, Japan
[2]Division of Molecular Bioregulation, Cancer Research Institute, Kanazawa University, Ishikawa, Japan

Recent data indicate that the deficiencies of several pro-inflammatory cytokine genes affect skin wound healing processes. Hence, we explored skin wound healing processes in mice deficient in IL-1 receptor antagonist (IL-1ra), a potent endogenous antagonist against a pro-inflammatory cytokine, IL-1. Wound closure was delayed with attenuated collagen accumulation in IL-1ra knockout (KO) mice, compared with wild-type (WT) mice. On the contrary, leukocyte recruitment was exaggerated with augmented IL-1 expression in IL-1ra KO mice, implying that IL-1ra deficiency enhanced local inflammatory reaction at the wound sites. Consistently, nuclear translocation of a pro-inflammatory transcription factor, NF-κB, was significantly enhanced and prolonged in fibroblasts in IL-1ra KO mice, compared with WT mice. Previous *in vitro* observations demonstrated that NF-κB activation could attenuate transforming growth factor (TGF)-β/Smad signaling pathway, which has crucial roles for collagen deposition in wound healing processes. Indeed, in IL-1ra KO mice, the TGF-β/Smad signaling pathway was suppressed as evidenced by decreases in phosphorylated Smad2/3 and a reciprocal increase in Smad7 at the wound sites, compared with WT mice. These results demonstrated that the absence of IL-1ra resulted in aberrant NF-κB activation and reciprocal diminution in TGF-β/Smad signaling and eventually attenuated collagen deposition during skin wound healing *in vivo*.

Rec.7/2/2007, 8/20/2007, pp31-35

* Correspondence should be addressed to:
Yuko Ishida, Department of Forensic Medicine, Wakayama Medical University, 811-1 Kimiidera, Wakayama 641-8509, Japan. Phone & Fax: 81-73-441-0641, e-mail: iyuko@wakayama-med.ac.jp

Key words wound healing, cytokines, IL-1ra, NF-κB, TGF-β1

Introduction

Healing processes of wounds start immediately as soon as tissue is injured and proceed with a complicated but well organized interaction among soluble mediators, extracellular matrix components, and various types of tissues and cells such as resident cells and infiltrating leukocytes[1] (Fig.1). Wound healing advances with a cascade of three phases consisting of inflammation, proliferation, and maturation. During inflammatory phase, neutrophils infiltrate at the wound within a few hours of injury and presumably protect the host from infection by combating invading microorganisms and clearing cellular debris. By 3 to 7 days after the injury, monocytes/macrophages dominate

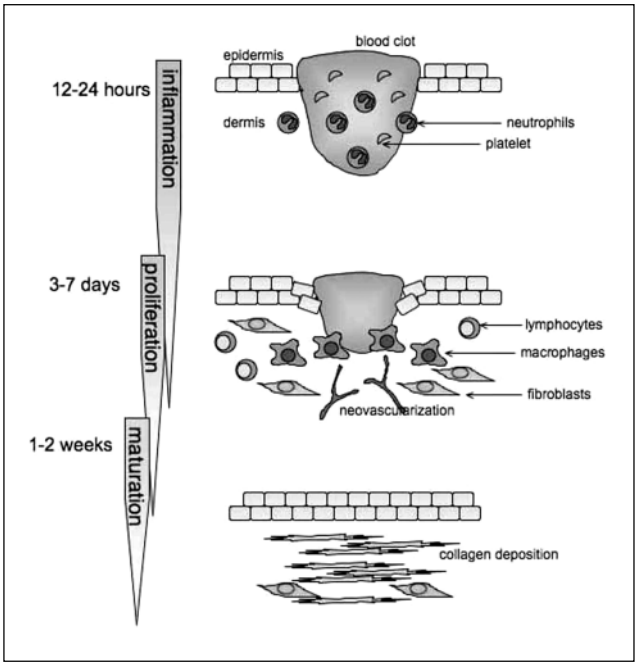

Fig.1 Skin wound healing processes

Skin wound healing starts immediately after an injury and consists of three phases; inflammation, proliferation and maturation. All of these stages are controlled by wide variety of cytokines and growth factors.

healing tissue and work as voracious phagocytes of matrix and cell debris including fibrin and spent neutrophils. In addition to these defensive activities, inflammatory cells are presumed to be also an important source of cytokines and growth factors, which initiate the proliferative phase. These observations proposed that inflammatory cells have beneficial roles in wound healing. In the proliferative phase, newly formed granulation tissue extends to complete repair with neovascularization, along with fibroblast and endothelial cell accumulation. All phases of skin wound healing processes are controlled by a wide variety of cytokines and growth factors. Therefore, the understanding of the dynamic and complex process of wound healing requires the clarification on the roles of each cytokine and growth factor involved in the processes.

Inflammatory cytokines in skin wound healing

We previously observed that the deficiencies of several proinflammatory cytokine genes profoundly affected skin wound healing processes (Table 1). When excisional skin wounds were generated in IL-6 KO mice, the reepithelialization and granulation tissue formation were significantly delayed with attenuated

Table 1 Inflammatory cytokines in mouse model of skin wound healing

	TNF-Rp55$^{-/-}$ (Ref. 3)	IL-6$^{-/-}$ (Ref. 2)	IFN-$\gamma^{-/-}$ (Ref. 5)	IL-1ra$^{-/-}$ (Ref. 9)
Leukocyte infiltration	↓	↓	↓	↑
Angiogenesis	↑	↓	↑	↓
Collagen deposition	↑	↓	↑	↓
Wound healing	↑	↓	↑	↓

leukocyte infiltration, compared with WT mice[2]. In contrast, tumor necrosis factor receptor p55 (TNF-Rp55) KO mice showed an enhanced angiogenesis and collagen accumulation, and eventually accelerated skin wound healing despite reduced leukocyte infiltration[3]. In TNF-Rp55 KO mice, gene expression of proinflammatory cytokines and adhesion molecules were reduced at the wound sites. However, growth factors such as TGF-β1, connective tissue growth factor (CTGF), and vascular endothelial growth factor (VEGF) were markedly enhanced at the wound sites in TNF-Rp55 KO mice[3].

Although interferon (IFN)-γ is a major factor produced by CD4$^+$ Th1 cells and acts on various types of immune cells, recent studies demonstrated that IFN-γ exerts multiple effects on non-immune cells, particularly fibroblasts. Several *in vitro* observations implied that IFN-γ inhibit TGF-β1 protein synthesis at a posttranslation level[4]. We also observed that skin wound healing was accelerated in IFN-γ KO mice with enhanced collagen deposition at the wound sites along with markedly enhanced TGF-β1 expression compared with WT mice[5]. Collectively, these inflammatory cytokines have essential roles in skin wound healing processes by regulating granulation tissue formation and/or leukocyte infiltration.

Leukocyte infiltration into skin wound sites

Leukocyte infiltration into the wounds has been thought to be indispensable for repair by producing various types of bioactive substances. On the contrary, Martin and colleagues demonstrated that PU.1-deficient mice exhibited effective skin wound healing without leukocyte infiltration[6]. Neutropenic mice exhibited accelerated wound closure upon an aseptic skin wound[7]. In contrast, the absence of secretory leukocyte protease inhibitor delayed skin wound healing despite or because of exaggerated leukocyte infiltration[8]. Indeed, IL-1 receptor antagonist (IL-1ra) KO

mice exhibited a delayed wound healing with an exaggerated neutrophil and macrophage infiltration[9]. We also demonstrated that IL-1ra KO mice exhibited retardation in the proliferative phase as evidenced by delayed granulation tissue formation and reduced angiogenesis[9]. Because these processes are governed with fibroblasts and endothelial cells, IL-1ra deficiency has profound effects on these resident cells in skin besides leukocytes. Moreover, we did not observe any correlation between leukocyte infiltration and wound healing rates of the aseptic skin wound in our various gene-deficient mice (Table 1)[2,3,5,9]. Thus, under the aseptic conditions, collagen deposition and angiogenesis can proceed in wound sites independently of leukocyte infiltration.

TGF-β1 and Smads in wound healing

TGF-β is found in large amount in platelets and is produced by several cell types that are present in a wound, including activated macrophages, fibroblasts, and keratinocytes. TGF-β1 KO mice exhibited decreased reepithelialization and decreased collagen deposition when compared with control mice[1]. Additionally, TGF-β is also essentially involved in scarring responses or keloid formation[1].

Collagen deposition is an indispensable step for wound healing. TGF-β1 has been implicated as a key mediator of collagen synthesis[10]. Upon binding, TGF-β1 activates the kinase activities of its receptor, which phosphorylates transcription factors, Smad2 and Smad3. Phosphorylated Smad2 and Smad3 associate with Smad4 and translocate into nucleus, thereby inducing the expression of the target genes. Simultaneously, Smad2 and Smad3 induce the expression of Smad7, which can interfere with the phosphorylation of Smad2 and Smad3 by ligand-bound TGF-β1 receptors[11]. Recently, several lines of evidence implied that the transcription of VEGF, a potent angiogenic factor, is also regulated in a TGF-β/Smads-dependent manner[12,13]. IFN-γ/Stat1 signaling pathway induces *in vitro* expression of Smad7, which prevents the interaction of Smad2/3 with the TGF-β receptor, resulting in the suppression of TGF-β-responsive genes[4]. Consistently, we observed increases in the amount of phosphorylated Smad2/3 at the skin wound sites in IFN-γ KO mice compared with WT mice[5]. Thus, the TGF-β1 signaling pathway was not inhibited in the absence of IFN-γ, which eventually resulted in acceleration of wound healing process[5].

IL-1 in wound healing

IL-1 is a representative proinflammatory cytokine that regulates many aspects of the immune and inflammatory responses. There are two IL-1 ligands with agonist activity, IL-1α and IL-1β, which are produced by various kinds of cells such as neutrophils, monocytes/macrophages and fibroblasts. Both IL-1α and IL-1β bind to the same receptor and have similar, if not identical, biological properties. IL-1α and IL-1β expression was strongly enhanced during wound healing[9]. Moreover, topical administration of IL-1α accelerated epidermal wound healing[14]. IL-1 receptor KO mice exhibited significantly delayed healing in oral but not dermal[15] and short-term but not long-term blockade of IL-1 can promote periodontal wound healing[16]. Thus, it still remains controversial on the roles of endogenously produced IL-1 in wound healing processes.

Roles of IL-1ra in skin wound healing *in vivo*

IL-1ra is also a member of IL-1 family and an endogenous antagonist against IL-1 by competitively blocking their binding to their receptors[17]. IL-1ra is aberrantly produced in various diseases such as rheumatoid arthritis and infectious diseases, to dampen the bioactivities of IL-1[17]. Although IL-1ra expression was enhanced at skin wounds[9], the role of IL-1ra in wound healing has not been investigated. To determine the pathophysiological role of the IL-1 system, particularly IL-1ra, in skin wound healing, we generated excisional wounds on IL-1ra KO mice[9]. Although there was no apparent differences in the wound areas at immediate and 1 day after wounding between WT and IL-1ra KO mice, the wound closure was markedly delayed in IL-1ra KO mice later then day 3 compared with WT mice (Fig.2a). Moreover, the enhancement of IL-1α, IL-1β, and TNF-α expression (Fig. 2 b-d), and leukocyte infiltration was exaggerated at the wound sites in IL-1ra KO mice, compared with WT mice.

A transcription factor, NF-κB, is sequestered in the cytoplasm with its inhibitor, IκB, in a resting state. Inflammatory cytokines such as IL-1 and TNF-α can induce the phosphorylation and subsequent degradation of IκB, followed by liberation of NF-κB and its nuclear translocation. In the nucleus, NF-κB dimers bind to target DNA elements and activate transcription of immune- and/or inflammation-associated genes, such as chemokines and adhesion molecules[18]. IL-1ra deficiency resulted in the enhancement of IL-1 and TNF-α expression and eventually the nuclear translocation of NF-κB was exaggerated and prolonged at the skin wound sites compared with WT mice[9] (Fig.2e). The net results are exaggerated augmentation in the expression of chemokines (MIP-2, KC and MCP-1) and adhesion molecules (ICAM-1 and VCAM-1) in IL-1ra KO mice compared with WT mice. This eventually resulted in enhanced leukocyte infiltra-

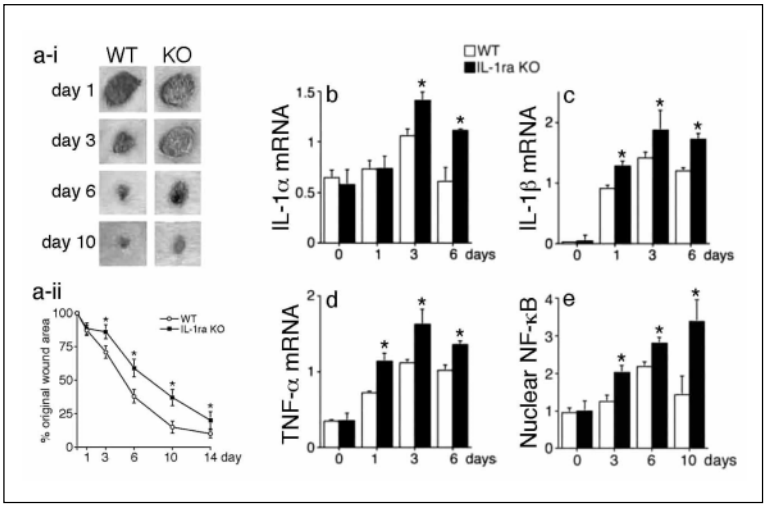

Fig.2

a: Macroscopic changes in skin excisional wounds in WT and IL-1ra KO mice. *a-i*: The wound sites were photographed at the time indicated. *a-ii*: Changes in percentage of wound area at each time point in comparison to the original wound area. *b-d*: mRNA expression of IL-1α (b), IL-1β (c), and TNF-α (d) at the wound sites of WT and IL-1ra KO mice. The ratios to β-actin are shown. *e*: The amount of nuclear NF-κB (p65) at the wound sites of WT and IL-1ra KO mice. Each value represents mean $\pm$ SEM (n = 6 animals). $*p < 0.05$, WT vs. IL-1ra KO mice. (Ref. 9 op.cit., Copyright 2006 The American Association of Immunologists, Inc.)

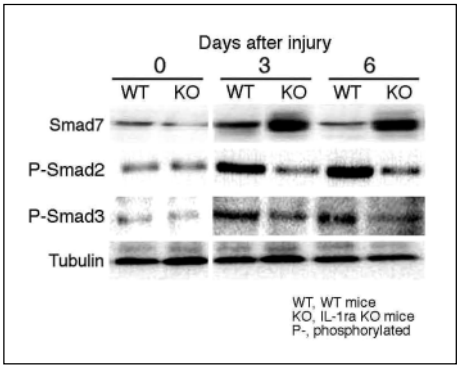

Fig.3

Western blot analysis for Smad7, phosphorylated (P)-Smad2 and P-Smad3 in the wound sites of WT and IL-1ra KO mice. Representative results from six individual animals in each group are shown. (Ref. 9 op.cit., Copyright 2006 The American Association of Immunologists, Inc.)

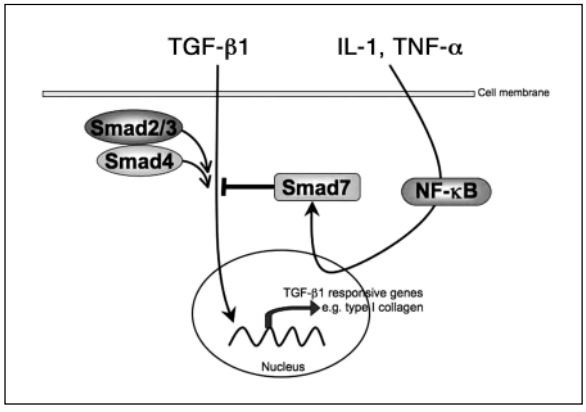

Fig.4

Schematic crosstalk between NF-κB and TGF-β1/Smad signaling in skin wound healing. In IL-1ra deficiency, NF-κB pathway regulates negatively TGF-β1/Smad pathway by inducing the expression of Smad7 and eventually reduce collagen production at wound sites *in vivo*.

tion at the wound sites. These observations indicated that absence of IL-1ra exaggerates the inflammatory responses at the wound sites via aberrant NF-κB activation[9].

NF-κB activation was evident in α-smooth muscle actin-positive myofibroblasts at the wound sites. Recent *in vitro* studies implied that the NF-κB pathway could negatively regulate TGF-β1/Smad signaling[19]. Consistently, the phosphorylated Smad2/3 were markedly reduced along with an increase in Smad7 proteins at the wound sites in IL-1ra KO mice compared with WT mice (Fig.3). This eventually led to suppressed gene expression of type I collagen and VEGF, target genes of TGF-β1/Smad signaling pathway[9]. Moreover, the depressed gene expression resulted in reduction in collagen accumulation and angiogenesis and eventually delayed wound healing in IL-1ra KO mice[9] (Fig.4).

Of interest is that TNF-Rp55 KO mice exhibited totally opposite phenotypes at skin wound sites, to IL-1ra KO mice[3]. Given the potent activities of TNF-α to activate NF-κB system, TNF-Rp55 deficiency can abrogate NF-κB-mediated inhibition of TGF-β1/Smad signaling pathway and accelerate wound healing processes with reduced infiltration of inflammatory cells (Fig.4).

Conclusions

An abnormal cytokine expression is frequently associated with impaired wound healing or scar formation, indicating that a correct temporal and spatial expression of these genes is essential

for normal healing. Our observations on IL-1ra KO mice suggest that IL-1ra may block the aberrant IL-1-mediated NF-κB activation and eventually augment TGF-β1 production and signaling pathway, thereby accelerating the healing processes of skin wounds. Thus, therapeutically, the blockade of the aberrant proinflammatory IL-1 by IL-1ra in wounds may accelerate healing, and could be beneficial in the context of surgery, chronic ulcers and other pathologic conditions.

Most efforts in wound healing research have been devoted to the research on growth factors, in order to find out efficient and novel therapeutic agents for patients with chronic wounds. However, because the biology of wound healing is much more complicated than initially assumed, the elucidation on the mechanisms underlying wound healing needs the consideration of additional components. Therefore, understanding the network of the signal transduction systems may give us more detailed insights into various soluble mediators including cytokines and growth factors.

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