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Review

Regulation of wound healing and organ fibrosis by toll-like receptors ☆

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ABSTRACT

Chronic injury often triggers maladaptive wound healing responses leading to the development of tissue fibrosis and subsequent organ malfunction. Inflammation is a key component of the wound healing process and promotes the development of organ fibrosis. Here, we review the contribution of Toll-like receptors (TLRs) to wound healing with a particular focus on their role in liver, lung, kidney, skin and myocardial fibrosis. We discuss the role of TLRs on distinct cell populations that participate in the repair process following tissue injury, and the contribution of exogenous and endogenous TLR ligands to the wound healing response. Systemic review of the literature shows that TLRs promote tissue repair and fibrosis in many settings, albeit with profound differences between organs. In particular, TLRs exert a pronounced effect on fibrosis in organs with higher exposure to bacterial TLR ligands, such as the liver. Targeting TLR signaling at the ligand or receptor level may represent a novel strategy for the prevention of maladaptive wound healing and fibrosis in chronically injured organs. This article is part of a Special Issue entitled: Fibrosis: Translation of basic research to human disease.

1. Introduction

The capacity to detect tissue injury and to initiate adequate repair mechanisms is indispensable for the survival of all higher species. A common aspect of all types of injury – caused by infectious, physical, chemical or immune processes – is a compositional change of the cellular environment leading to the presence of novel molecular patterns. These patterns are recognized by a group of receptors termed pattern recognition receptors (PRRs), and trigger specific responses that promote the restoration of tissue function, including inflammation and wound healing.

Pathogen recognition is critical for survival in an essentially hostile environment that is full of potentially infective microorganisms. Detection systems for molecular patterns characteristic for pathogens (*pathogen-associated molecular patterns* = PAMPs) developed early in evolution, and are present in most species including plants and invertebrates [1,2]. Toll-like receptors (TLRs) are a group of highly conserved pattern recognition receptors that signal the presence of various PAMPs to cellular constituents of the innate and adaptive immune system. Upon binding to distinct biochemical components of protozoa, bacteria and viruses, TLRs trigger immune responses via NF-κB-dependent and interferon regulatory factor (IRF)-dependent mechanisms. In addition to their function as pathogen recognition receptors, TLRs may also be activated by endogenous ligands termed *damage-associated molecular patterns* (DAMPs), which are either inaccessible to the immune system under physiologic conditions or undergo changes in response to injury, leading to recognition by PRRs. After tissue injury, these patterns are unmasked or released from damaged cells, and subsequently trigger inflammation via TLRs and other PRRs. Accordingly, TLRs can be considered as master safeguards of the structural integrity of tissue: activated through molecular indicators of infection or injury, they exert a key role in initiating countermeasures that repair the wound and protect the host from further damage.

Inflammation and wound healing are tightly linked processes as demonstrated by the need for inflammatory signals to recruit both fibroblasts and macrophages for tissue repair and removal of debris. However, sustained inflammation may lead to maladaptive processes like loss of functional parenchyma, fibrosis and carcinogenesis in a variety of organs [3–5] (see Fig. 1). TLR signaling in particular has been identified as a trigger of inflammation leading to dysfunctional wound healing in several chronic diseases [5]. Here, we will review the role of TLRs in the regulation of wound repair and fibrogenesis in the setting of chronic injury, with a particular focus on five main organs, which are commonly affected by chronic inflammation and maladaptive wound healing responses: the liver, kidney, lung, skin and heart. Insight into the complex interactions between TLRs and their ligands may reveal novel targets for the prevention or treatment of these maladaptive responses to chronic injury.

2. Wound healing and the danger hypothesis

Despite being sterile, tissue injury often leads to profound inflammation [6]. Inflammatory, fibrogenic and regenerative responses are

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part of a complex and intertwined injury response system that serves to contain damage and to restore and maintain tissue function through recruitment of several types of specialized cells: platelets and fibroblasts provide mechanical stability to the wound in short and long terms, phagocytic leukocytes combat potential pathogens and clear the site of injury from dead cells and debris, and progenitor or stem cells replace functional epithelium. Inflammation may be initiated via acellular biochemical reaction cascades such as the complement system and coagulation cascade, and via the activation of resident immune cells. Both lead to the recruitment of circulating white blood cells into the injured tissue and the production of different cytokines, which further amplify inflammation and cell recruitment, or regulate important aspects of tissue repair such as regeneration and fibrogenesis. The inflammatory phase of wound healing resolves within several days and transitions into a regenerative phase, characterized by extracellular matrix (ECM) production through activated and proliferating fibroblasts, as well as angiogenesis, re-epithelialization and wound contraction. Ultimately, the provisional matrix remodels into the "pre-injury state", or matures into scar tissue.

Numerous mediators that are essential for inflammation, wound healing and fibrogenic responses are well-established including inflammatory cytokines, TGFβ, PDGF and several chemokines [7]. However, it is not well understood through which mechanisms cells sense the presence of tissue injury and initiate these responses in the first place. One hypothesis is that the activation of the coagulation cascade following damage of vascular endothelial cells is a major stimulus for wound healing and inflammation, as demonstrated by proinflammatory and profibrogenic effects of cleaved clotting factors on tissue-resident and circulating cells [8–11], as well as the release of fibrogenic mediators such as serotonin, PDGF and TGFβ from activated platelets or endothelial cells [7,12–14]. A second hypothesis is that the encounter with unknown molecular patterns released from damaged or injured cells (DAMPs) triggers inflammatory, fibrogenic and regenerative responses in specific cell types [15–17]. Finally, physical changes in the extracellular environment such as an increase in pressure and tissue stiffness may also trigger or amplify fibrosis via activation of fibrogenic cell populations through mechanoreceptors [18–20]. These hypotheses

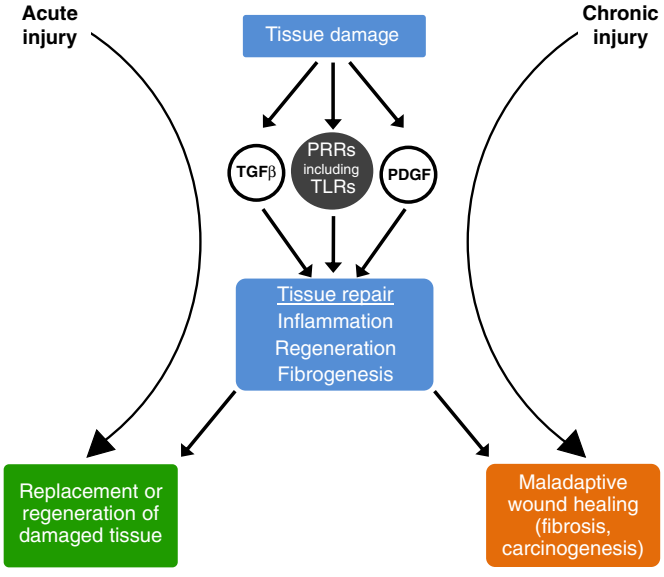

Fig. 1. Central role for pattern recognition receptors (PRRs) in wound healing responses. After tissue damage, molecular indicators of injury or stress activate pattern recognition receptors to induce measures that contribute to the restoration of tissue integrity. Chronic injury may trigger maladaptive wound healing through the same effector systems, and ultimately lead to fibrosis and cancer.

are not mutually exclusive, and it is likely that the coagulation cascade, DAMPs and physical forces co-operatively regulate wound healing responses.

DAMPs are a growing group of signature molecules comprising biochemical entities as diverse as nucleic acids, extracellular matrix fragments, cytoskeleton components, small molecules like uric acid and ATP, as well as large proteins such as heat shock proteins (HSPs), S100 proteins or high mobility group box protein 1 (HMGB1) [21–26]. Under physiologic conditions, these molecules do not trigger inflammation due to seclusion in intracellular compartments or physicochemical properties that render them inert to PRRs. After tissue injury, however, DAMPs are either released into the extracellular space or undergo chemical changes, leading to recognition by PRRs expressed by immune cells and other cell types. Based on these insights, it has been suggested that the immune system has evolved not only to detect patterns that signal foreignness, but also to globally detect patterns that signal danger [27]. In the setting of chronic injury, DAMPs and their receptors may contribute to maladaptive wound healing by triggering chronic inflammation and activation of fibrogenic cell populations [5,28,29].

3. Toll-like receptors and signaling

Toll, the founding member of the TLR family, was described more than 20 years ago as a regulator of dorsoventral polarization processes in the embryogenesis of *Drosophila melanogaster* [30]. Subsequently, a vital role of Toll in the induction of an immunological defense against fungal and bacterial infections was uncovered, and homologous receptors in vertebrates were identified [31–33]. In contrast to *Drosophila*, vertebrate species exhibit a diversification of TLRs up to 12 different transmembrane proteins, allowing the detection of a wide range of ligands. In combination and in cooperation with other pathogen recognition receptors, TLRs precisely exert the function of pathogen recognition, which was postulated by Charles Janeway more than 20 years ago [34]. As conserved germline-encoded pattern recognition receptors, TLRs empower cells of the innate immune system to reliably differentiate "self" from "nonself" patterns through detection of conserved microbial PAMPs. The recognized patterns are unique to microbes and essential products of their metabolism, so that mutations cannot readily render these organisms undetectable by TLRs. Finally, the interactions between PAMPs with TLRs are perceived as signatures of infection and consequently induce an immunological response.

3.1. TLR ligands

TLRs efficiently recognize distinct components of various pathogens through direct or adaptor-mediated binding (see Table 1): TLR1, TLR2 and TLR6 recognize cell wall components of gram-positive bacteria such as peptidoglycan, N-acyl lipoproteins or lipoteichoic acid. TLR3 and TLR7 are activated by double- or single-stranded RNA, indicating viral infection, and TLR9 senses unmethylated CpG dinucleotides, a characteristic feature of bacterial and viral pathogens. TLR4 recognizes distinct components of lipopolysaccharide (LPS), a cell wall component of all gram-negative bacteria that consists of a lipophilic region ("lipid A"), a covalently linked hydrophilic oligosaccharide and a polysaccharide chain termed *O-polysaccharide* [35,36]. Lipid A constitutes the biologically active compound of LPS, whereas the presence ("smooth" LPS) or absence ("rough" LPS) of *O-polysaccharide* varies depending on the microbial source of LPS and determines the need for additional adaptor molecules for TLR4 activation [36,37].

Importantly, most TLRs may be activated by diverse ligands, which often do not share any obvious structural similarities. It is therefore believed that TLRs may have a wide ligand spectrum that possibly includes patterns not derived from pathogens [38,39]. This concept is exemplified by hyaluronic acid, a major component of the extracellular matrix in virtually all organs. In its physiological, unfragmented form ($M_w > 1000$ kDa), hyaluronan is biologically

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