REVIEW ARTICLE

Role of IL-18 in transplant biology

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ABSTRACT. Since pro-inflammatory cytokine IL-18 and its receptor (IL-18R) are closely involved in regulating both adaptive and innate immune responses, it is conceivable that they might play an important role in organ transplantation. IL-18 can stimulate lymphocytes to produce the IFN-γ and regulate macrophage activity, thereby increasing the expression of proinflammatory cytokines including IL-1β, IL-6, CCL4 (macrophage inflammatory protein-1 β), CXCL2 (macrophage inflammatory protein-2), and CCL2 (monocyte chemotactic protein-1). Nevertheless, the IL-18 signaling pathway and its underlying mechanisms remain obscure in transplant biology. This review is to summarize recent advances in our knowledge about the IL-18 signaling pathway and to analyze their functions in transplant-related biology. It was found that IL-18/IL-18R signaling pathway contributed to vascular transplantation, ischemmia/reperfusion, acute kidney injury, and acute rejection of kidney/liver/heart transplantation. IL-18 was a potential CYP3A expression modulator and was capable of affecting tacrolimus pharmacokinetics. Neutralizing IL-18 by its inhibitor IL-18 binding protein could efficiently suppress the production of injury-associated cytokines such as IL-6, TNF-α, IFN-γ, CXCL10 (IFN-γ-inducible protein10), and CX_3CL1 (fractalkine) and improve allograft function. Blockade of IL-18 signaling could regulate cardiomyocyte apoptosis and inhibit Th17 cells differentiation. Alteration of IL-18 levels was suggested as a biomarker for predicting ongoing allograft outcome. All these activities could deepen our understanding of immunobiological role of IL-18 and its receptor in the field of organ transplantation. Intervention of IL-18 signaling pathway might be utilized as a therapeutic strategy in clinic.

Key words: IL-18, inflammatory cytokines, organ transplantation

Interleukin-18 (IL-18) is a member of the interleukin-1 family. It is initially called IFN-γ (interferon-γ) inducing factor and plays an important role in both innate and adaptive immunobiology [1]. IL-18 receptor (IL-18R) is expressed on Th1 lymphocytes and, therefore, IL-18 is capable of potently inducing Th1 responses [2]. In addition, IL-18 bearing pleiotropic effects can induce Th2 responses with no need of IL-12 [3]. Boost of Th1 and Th2 immune responses can elicit various cytokines including IL-1β, IL-2, TNF-α (tumor necrosis factor-α), IFN-γ, adhesion molecules, and apoptosis factors (*figure 1*) [4-6]. IL-18 can be expressed by a wide range of immune cells (T cells, B cells, NK-T cells, neutrophils), although the main source of IL-18 is from the activated macrophages [2]. Other non-immune cells can also express IL-18 such as intestinal and airway epithelial cells, keratinocytes, airway epithelium, corneal epithelial cells, renal tubular epithelial cells [7]. The main role of IL-18 is to crucially stimulate lymphocytes to produce the IFN-γ [5] and regulate macrophages activity, thereby increasing the expression of pro-inflammatory cytokines including IL-6, IL-1β, CCL4 (macrophage inflammatory protein-1 β), CXCL2 (macrophage inflammatory protein-2), and CCL2 (monocyte chemotactic protein-1) [8]. IL-18 in conjunction with IL-12 can stimulate the production of IFN-γ by B and T cells and potentiate the cytolytic activity of natural killer (NK) cells, and induce Th1 and Th2 responses (*figure 1*) [9, 10]. In addition, IL-18/IL-1β signaling pathway can trigger the transcription of multiple inflammatory genes via the activation of nuclear factor κB and transcription activator-1. Subsequently, pro-inflammatory cytokines, chemokines, adhesion molecules, and colony-stimulatory factors are induced to enhance leukocyte infiltration [11]. These aforementioned cytokines are capable of promoting acute cellular rejection in solid organ transplantation. Strikingly, damage caused by IL-18 does not depend upon CD4+T cells or neutrophils [12].

Over-production of IL-18 can potently cause a variety of severe inflammatory disorders such as autoimmune diseases, transplant rejection, and ischemia/reperfusion injury [13]. In clinic, it was observed that IL-18 was closely

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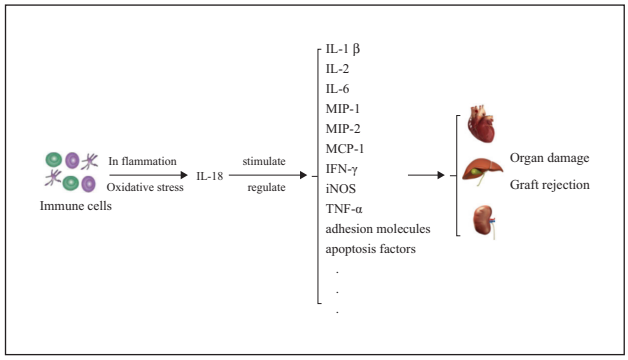

Figure 1
Scheme for the role of interleukin (IL)-18 in the elicitation of oxidative stress and proinflammation causing organ damage and graft rejection in organ transplantation.

associated with various human diseases such as inflammatory bowel disease, systemic lupus erythematosus, sub-acute sclerosingpanencephalitis, rheumatoid arthritis, and allograft dysfunction posttransplantation [13]. Inhibitors of IL-18 (rIL-18BP (binding protein), GSK 1070806, ABT-325) were successfully developed for clinical studies especially for chronic inflammation scenarios [14]. This present review attempts to analyze the roles of IL-18 signaling pathway in transplant biology, which may shed light on unveiling allogeneic immunoresponses and transplant tolerance induction.

ROLE OF IL-18 IN VASCULAR TRANSPLANTATION

Both IL-18 and IL-1β belong to the same family of IL-1. They have a homologous amino acid sequence and share specific biological functions [15]. As important pro-inflammatory cytokines, both of them are closely involved in the pathophysiology of vein graft remodeling [11]. As a vein was engrafted into an artery, vascular smooth muscle cells (VSMCs) turned massively necrotic and monocytes infiltrated into venous wall of graft. Afterwards, IL-18 and IL-1β were highly expressed by monocytes at 1 week after vein transplantation [11]. It was found that peripheral level of IL-18 was closely associated with carotid intima-media thickness in clinic [16]. Experimental study exhibited that neutralizing IL-18 and IL-1β by using antagonist recombinant IL-1ra-Fc-IL-18bp could effectively suppress the activation of VSMC proliferation, cell growth–related signaling molecules, and vein graft thickening in vivo [11].

ROLE OF IL-18 IN ACUTE KIDNEY INJURY (AKI)

Acute kidney injury (AKI) is a relatively common and serious complication after liver transplantation or ischemia/reperfusion in clinic [17, 18]. Serum IL-18 as a proinflammatory cytokine is closely associated with inflammatory events and multi-organ dysfunction [19]. Liver ischemia and reperfusion (1 hour and 4 hours respectively) could induce expression of IL-18, IL-6, IL-1β, and TNF-α, leading to renal damage [18]. It was observed that serum IL-18 and urine IL-18, IL-6, CXCL8 (IL-8), and NGAL levels in patients were significantly increased in AKI within the first 24 hours after liver transplantation [17]. The alteration of urine and the serum level of interleukin-18 were involved in ischemia-reperfusion injury and acute kidney ischemia, suggesting that they could be utilized as an early predictor for acute kidney injury (AKI) after liver transplantation [19]. Indeed, pretreatment with IL-18BP could attenuate renal damage with antioxidant and anti-inflammatory effects [17, 18]. Recently, clinical data displayed that combined use of IL-18 and endothelin-1 would better predict AKI at the early stage after liver transplantation [20].

ROLE OF IL-18 IN KIDNEY TRANSPLANTATION

Experimental studies exhibited that IL-18 had a key role in rat and mouse kidney transplantation. In a mouse MHC-mismatched model of acute renal rejection, IL-18 and its receptor expressions were dramatically upregulated within kidney allografts, whereas IL-18 and its downstream pro-inflammatory cytokines (iNOS, TNF-α, IFN-γ) expressions were significantly downregulated in IL-18$^{-/-}$ recipients (*figure 1*) [21]. In a rat model of acute kidney allograft rejection, a 3-fold upregulation of IL-18 mRNA expression was detected within allografts, which were mainly from ED1^{+} macrophages [22]. Similar clinical observation was achieved, in which a significant elevation of serum IL-18 (approximately 3-fold) was found in patients with kidney rejection [23]. Further investigation revealed that epithelium of distal tubules, proximal tubules, and infiltrating leukocytes in the kidney expressed IL-18 mRNA and released mature IL-18 particularly in response to IFN-γ and TNF-α [23]. In clinic, urinary IL-18 levels were apparently augmented in patients with acute tubular necrosis (ATN) and delayed graft function, therefore, which was suggested as a biomarker for proximal tubular injury in ATN [24]. A significantly increased concentration of IL-18 in urine was observed, which was considered as a biomarker for acute renal failure [5]. IL-18 in combination with NGAL (neutrophil gelatinase-associated lipocalin) was suggested as a biomarker for delayed graft function after kidney transplantation [25]. Subsequently, other researchers further confirmed these findings in clinic. Multivariate analysis exhibited that augmentation of IL-18 or NGAL might predict a need for ongoing dialysis within first week of kidney transplantation after adjusting for recipient and cold ischemia time, donor age, urine output, and serum creatinine. In addition, IL-18 and NGAL quantiles could accurately predict graft recovery up to three months later [25].

Past studies revealed that IL-18 (rs187238) gene polymorphism was not associated with kidney graft outcome after transplantation [5]. The -137C/G (rs187238) and -607C/A (rs1946518) variant alleles in the IL18 gene were not associated with creatinine clearance, implying that IL-18 polymorphisms could not affect kidney function [26]. Nevertheless, it was found that an increase of frequency of the IL-18 major haplotype -607C/-137G was observed in renal transplant patients [26]. Presence of AA genotype of IL-18 (rs1946518) was indicative of 2.35 higher risk of chronic rejection occurrence [5].

In addition, both IL-18 and IL-1β were potential CYP3A expression modulators. In kidney transplantation, CYP3A-dependent drug disposition might be influenced by IL-18 variability in different recipients [4]. Furthermore, IL-18 was involved in the induction of various inflammatory cytokines, which also could downregulate cytochrome P450 enzyme activities and influence CYP450-dependent drug disposition. Tacrolimus as an immunosuppressant is commonly used in kidney transplantation. It was found that its concentration/dose (C/D) ratio was evidently associated with IL-18 rs1946518 gene polymorphism in the first month after kidney transplantation. IL-18 promoter polymorphisms might facilitate to individualize tacrolimus treatment based upon CYP3A5 genotype [4].

ROLE OF IL-18 IN LIVER ISCHEMIA-REPERFUSION INJURY

Ischemia-reperfusion is an inevitable process for organ transplantation, in which blood supply is restored and subsequently free oxygen radicals and proinflammatory cytokines are induced to result into graft injury. IL-18 is one of the insulting cytokines mainly responsible for producing IL-1β and TNF-α from mononuclear cells. In hepatic ischemia-reperfusion injury model, Kupffer cells were main source of IL-18 at early stage. Neutralizing IL-18 by its inhibitor IL-18 binding protein (BP) could efficiently alleviate hepatic function and inhibit generation of injury-associated cytokines such as IL-6, TNF-α, and IL-18. Administration of IL-18BP mitigated hepatic oxidative stress, which was well-recognized to trigger production of pro-inflammatory cytokines and cell adhesion molecules. Afterwards, hepatic pathological structure was protected [8].

ROLE OF IL-18 IN LIVER TRANSPLANTATION

In a rat liver transplant model, overexpression of IL-18BP by using adenovirus gene transfer (Adex-IL18bp) could significantly decrease serum alanine aminotransferase levels and prevent histologic hepatic injury in transplant recipients. The underlying mechanisms were that IL-18BP specifically blocked the binding of mature IL-18 to its receptor and thereby suppressed IL-18-induced IFN-γ production. Pre-treatment with Adex-IL18bp caused a significant prolongation of rat liver allograft survival with a lower expression level of IFN-γ, CXCL10 (IFN-γ-inducible protein10), and CX_3CL1 (fractalkine) [7]. However, no significant association between IL-18 (-656G/T, rs1946519) and acute rejection of liver transplant patients was observed in clinic. As liver transplant patients were subcategorized, it was found that IL-18 TG genotype had a remarkable association with rejection in female patients compared with males. Therefore, the genotype of IL-18 TG was a sex-dependent risk factor for acute rejection episode of liver transplantation [13]. Furthermore, it was observed that IL-18 (rs1946519) expression was increased for insulin resistance posttransplantation, which might be novel important biomarker for diabetes mellitus after liver transplantation [27].

In addition, it was found that IL-18 was associated with tacrolimus pharmacokinetics. One Chinese research group utilized HRM analysis (high-resolution melting curve analysis) to study two single-nucleotide polymorphisms G-137C (rs187238) and A-607C (rs1946518) in the promoter region of IL-18 gene for tacrolimus pharmacokinetics and hepatic allograft dysfunction among human 150 liver transplant recipients. These two single nucleotide polymorphisms (A-607C and G-137C) were repeatedly associated with IL-18 promoter transcription activity. The findings indicated that IL-18 decreased tacrolimus concentration/dose (C/D) ratio post-transplantation. Higher IL-18 and lower tacrolimus concentration/dose (C/D) ratio were indicative of the risk of subsequent hepatic allograft dysfunction [6].

ROLE OF IL-18 IN HEART TRANSPLANTATION

In a mouse heart transplant model, lower inflammatory cytokines such as IL-1β, IL-17, IL-23, and IL-18 were detected in the IL-18BP-treated mice. Th17 differentiation was suppressed in vitro and in vivo. Adoptive transfer of T cells from IL-18 binding protein treated mice could ameliorate cardiac ischemia/reperfusion myocardial injury and cardiomyocyte necrosis. Infiltration of CD4+T cells, neutrophils, and macrophages was then prevented on 24 h after reperfusion. In addition, blockade of IL-18 signaling could regulate cardiomyocyte apoptosis. These findings manifested a destructive role of inflammatory cytokine IL-18 in cardiac IR injury and heart transplantation [2].

CONCLUSIVE REMARKS

Neutralizing IL-18 by its inhibitor IL-18 binding protein could suppress the production of injury-associated cytokines and Th17 cells differentiation and improve allograft function. Alteration of IL-18 level might be indicative of ongoing acute kidney injury, diabetes mellitus, and allograft outcome. All these aforementioned data could deepen our understanding of immunobiological role of IL-18 and its receptor in the field of transplantation. Intervention of IL-18 signaling pathway might be utilized as a therapeutic strategy in clinic.

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