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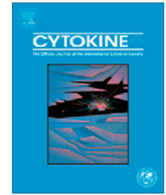

Interleukin-10 activates Toll-like receptor 4 and requires MyD88 for cardiomyocyte survival

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ABSTRACT

Toll-like receptors (TLRs) are important in a variety of inflammatory diseases including acute cardiac disorders. TLR4 innate signaling regulates the synthesis of anti-inflammatory cytokine, interleukin-10 (IL-10) upon TLR4 agonists' re-stimulation. Anti-apoptotic action of IL-10 in cardiac dysfunction is generally accepted but its protective mechanism through TLR4 is not yet understood. We studied the effect of IL-10 in the activation of TLR4 downstream signals leading to cardiomyocytes survival. IL-10 caused a significant increase in the expression of CD14, MyD88 and TLR4. TLR4 activation led to the translocation of the interferon regulatory factor 3 (IRF3) into the nucleus. Phosphorylation of IRF3 enhanced mRNA synthesis for IL-1β but not TNF-α and was elevated even after removal of IL-10 stimulation. Furthermore, degradation of inhibitory kappa B (IκB) kinase (Ikk) suggested that IκBβ was the main activating kinase for IRF3-regulated NF-κB activation and phosphorylation of p65. Phosphorylated NF-κB p65 was translocated into the nucleus. Concomitantly, an increase in Bcl-xL activity inhibited Bax and the proteolytic activity of caspase 3 as well as a decrease in PARP cleavage. An inhibition of MyD88, modulated the above listed responses to IL-10 as there was a decrease in TLR4 and IRF3 and an increase in TNF-α mRNA. This was associated with a decrease in NF-κB p65, Bcl-xL mRNA and protein levels as well as there was an activation of Bax and PARP cleavage independent of caspase 3 activation. These data in cardiomyocytes suggest that IL-10 induced anti-apoptotic signaling involves upregulation of TLR4 through MyD88 activation.

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1. Introduction

The pro-inflammatory cytokine, tumor necrosis factor alpha (TNF-α) has been shown to be upregulated in conditions of myocardial dysfunction and heart failure [1–3]. In a rodent model of myocardial infarction, we have earlier reported that there is a significant increase in TNF-α and a decrease in anti-inflammatory cytokine interleukin-10 (IL-10) [4]. Further, it has been shown in isolated cardiomyocytes that TNF-α causes a significant increase in pro-apoptotic proteins, apoptosis and membrane leakage [5,6] and that IL-10 antagonizes these TNF-α induced changes [6]. Recently, it has been reported that IL-10 mitigates the effect of TNF-α and cuts off the apoptotic signal generated by inhibitory kB kinase (Ikk) [7]. Very recently, we have also reported that when IL-10 bind to its receptor, it activates pro-survival signal via activation of Jak/Stat3 pathway [8] but details of its innate response are still unclear.

Anti-inflammatory property of IL-10 may be regulated through its innate signaling via activation of patterns recognition receptors such as Toll-like receptors (TLRs). Toll-like receptors have been shown to be the first line of host defense against microbial infection and play a central role in innate as well as adaptive immunity [9–11]. TLRs are also capable of responding to stress and modulate inflammation as well as tissue damage following non-infectious conditions such as hypoxia and ischemia in cardiac tissues [12–14]. Among 10 TLRs identified in humans, TLR2 and TLR4 have been reported during myocardial infarction [15] and ischemia reperfusion [16]. TLR2 activation by the agonist peptidoglycan-associated lipoprotein (PAL) or LTA is also reported to cause cardiac inflammation as well as dysfunction [17]. TLR4 agonist is directly

Abbreviations: Anti-IL-10R, antibody to interleukin-10 receptor; CD14, cluster differentiation 14; Ikk, antibody to interleukin-10 receptor; IkkB, inhibitory kinase B; IL-1β, interleukin-1β; IL-10, interleukin-10; IRF3, interferon regulatory factor 3; ISRE, interferon-sensitive response element; LBP, lipid binding protein; LPS, lipopolysaccharide; MyD88, myeloid differentiation factor (88); NF-kB, nuclear factor-kappaB; PAMPs, pathogen-associated molecular patterns; PARP, poly (ADP-ribose) polymerase; TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor-α.

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transferred into phospholipid bilayer, and via co-receptor CD14 activates MyD88 dependent TLR4 downstream signals [18,19]. Mechanism of MyD88 dependent signaling is based on TLR4 agonist stimulation and its signal strength. TLR4 has an absolute requirement of NF-κB [20] and in this process, phosphorylation of subunit p65 occurs when the interferon-sensitive response element (ISRE) complexes with Interferon regulatory factor 3 (IRF3) [21]. Thus, MyD88 and IRF3 synergistically activate NF-κB p65 [22–24]. TLR4 ligands trigger activation of cell survival as well as some inflammatory genes via NF-κB signaling. TLR4 innate signaling [25] also induces genes to regulate the synthesis of cytokines [22,26]. Involvement of TLR4 in the activation of IL-10 has also been reported as TLR4 agonist-mediated signals led to the synthesis of endogenous IL-10 upon re-stimulation of LPS [27]. On the other hand, endogenous production of IL-10 showed a critical role in myocardial ischemia/reperfusion injury [28]. Thus there is a possible interplay between TLR4 and IL-10.

In this study, we have examined events downstream to IL-10 activation of TLR4, which requires the intracellular adaptor molecule MyD88 in the cell survival signal in isolated cardiomyocytes. LPS stimulation was used as a positive control.

2. Materials and methods

2.1. Chemicals

Anti-rabbit TLR4, anti-rabbit PARP, anti-rabbit Bax, anti-rabbit Bcl-xL, anti-mouse MyD88 and anti-mouse caspase 3 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, California, USA). Recombinant IL-10 and anti-phospho and/or total NF-κB p65, IKK (α, β and ε), IRF3, TNF-α and IL-1β were also purchased from Cell Signaling Technology (USA). Anti-IL-10 receptor (anti-IL-10R) antibody (clone 1B1.3a) was purchased from BD Pharmingen, USA. HRP labeled anti-mouse or rabbit IgG and anti-biotinylated antibodies were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). FITC labeled CD14 antibody, and MyD88 homodimerization peptide RDVLPGT inhibitor were purchased from Calbiochem (La Jolla, California, USA). RNA isolation kit was purchased from Sigma (Sigma, USA), SYBR Green one step qRT-PCR kit from Quanta BioScience (USA) and PCR primers [29,30] for GAPDH, (sense: 5′TGC ACCACCAACT GCTTAGC 3′ and anti-sense: 5′GGCATGGACT GTGGTC ATGAG3′); NF-κB, (sense: 5′AAGAT CAATGGCTACACGGG3′ and anti-sense 5′ATCTTGAGCTCGGCAGTGTT3′) TNF-α, (sense: 5′CCTCTTCT

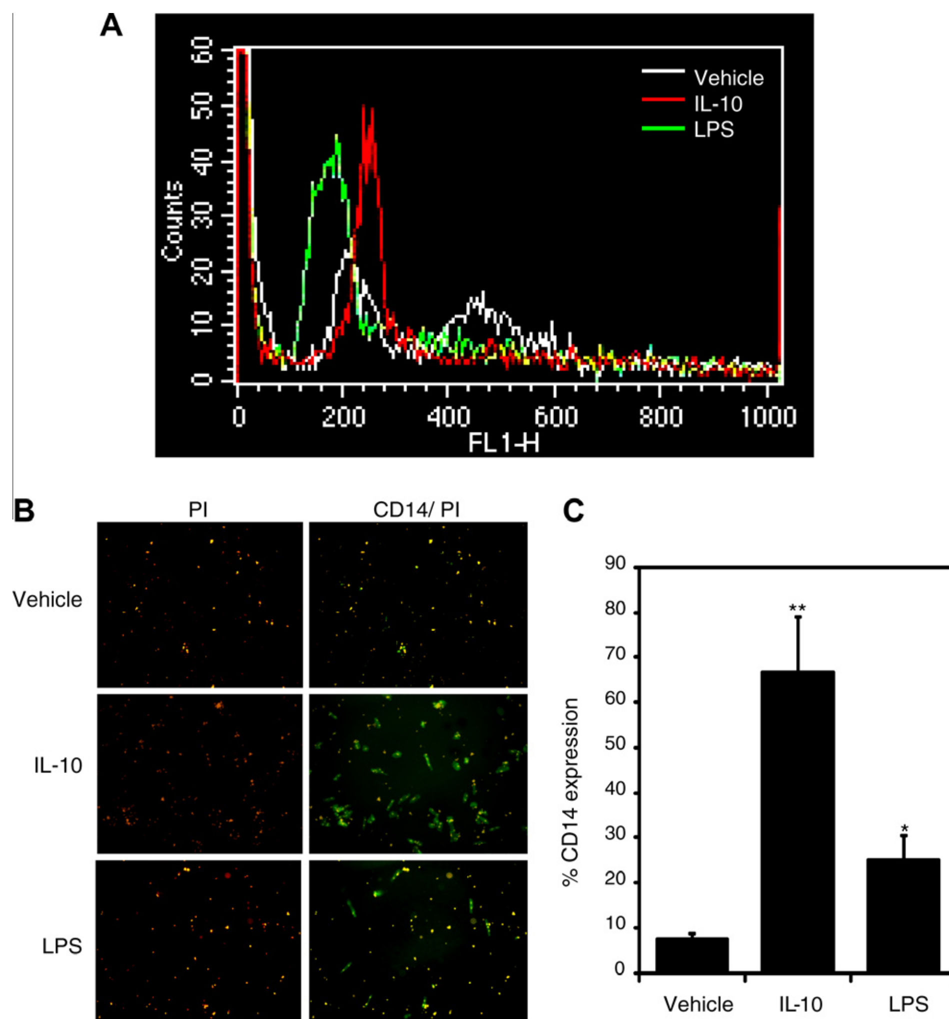

Fig. 1. CD14 expression in isolated cardiomyocytes. (A) *Flowcytometry*: a typical single-parameter histogram selected from five independent experiments done in duplicate, for CD14 expression in cardiomyocytes after 18 h stimulation with either 10 ng/ml of IL-10 (red) or 1 μg/ml of LPS (green) or PBS (vehicle; white); (B) immunocytochemistry and (C) percent expression of IL-10 or LPS induced CD14 expression (green) in cardiomyocytes. Nuclear staining was done using Propidium Iodide (orange). $^{*}P < 0.05$ vs. vehicle control and $^{**}P < 0.05$ vs. LPS as well as vehicle.

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