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The Role of IL-27 as An Anti-Inflammatory in A Severe Burns Model

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Abstract

Activation of IL27 can bind to one of its receptors gp130. This bond is able to regulate the signaling pathway and SOCS3 which plays a role in inhibiting the activation of proinflammatory cytokines such as TNF. In the case of burns, damaged cells potentially secrete the excessive pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1. These cytokines induce a SIRS which leads to sepsis causing multiple organ damage and death. This research aimed at proving the role of IL-27 as an anti-inflammatory to reduce TNF-α expression after severe burns. In this research I used laboratory experimental method. The research design was divided into 3 groups. The dorsal surfaces of mice were shaved to create a full-contact burn on approximately 28% of TBSA. The results of this research proved that IL-27 served as an anti-inflammatory agent through macrophage regulation to inhibit NF-kB activation because IL-27 has a gp 130 receptor which can induce the STAT3 signaling pathway and SOCS3.

***Key words**: Interleukin-27, Burn Injury, TNF-α, SOCS3*

Introduction

Interleukin-27 (IL-27) is a heterodimeric cytokine consisting of Epstein-Barr virus-induced gene 3 (EBI3) and IL-27p28, this cytokine binds to its receptor gp130[(1)]. gp130 can induce the JAK / STAT signaling pathway to SOCS3[(1)(2)(3)(4)]. In a study Wang et al, STAT3 acts as an anti-inflammatory by suppressing NFkB to secrete pro-inflammatory cytokines and chemokines[(5)]. In addition, SOCS3 activated by STAT3 acts as a negative regulator by inhibiting the NFkB signaling pathway[(6)]. These two proteins significantly inhibit the secretion of pro-inflammatory cytokines, one of which is TNF-α.

In the case of burns, damaged cells have the potential to secrete excess pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1. These cytokines induce a SIRS which leads to sepsis leading to multiple organ damage and death[(7)]. The objective of this study was to prove the role of IL-27 as an inducer of anti-inflammatory molecules so as to reduce TNF-α expression after severe burns. We postulated that IL-27 was able to regulate macrophages to inhibit TNF-α cytokine secretion.

Materials and Methods

Healthy female Balb-C mice (aged 8-9 weeks, weighing 20-25 g) were obtained from Animal Laboratories, Brawijaya University. Recombinant IL-27 (carrier free) was obtained from Biolegend (San Diego, CA). They were fed with a standard rodent chow diet and watered and housed with a 12 h light/dark cycle. All animals were allowed to acclimate for 1 week prior

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to the experiment and randomly divided into 3 groups: normal (no burn), burn, and burn + rIL-27. Mice were anesthetized by intraperitoneal (i.p.) injection of xylazine and ketamine. Their dorsal sufaces were shaved to create a full-contact burn on approximately 28% of total body surface area (TBSA). A 65-g rod copper rod (1.2 cm in diameter), heated to 90°C was used. The rod was applied four times for 8 seconds respectively in different spots of the animal's dorsal/flank to inflict an injury. Those animals were returned to individual cages, fed and watered *ad libitum*, and monitored three times a day. Immediately after burn in the burn and burn + rIL-27 groups, the mice were treated with 0.5 ml of 1% BSA and rIL-27 (10 µl/mice in 0.5 ml of 1% BSA, Biolegend) respectively. One day post injury, all animals were then killed and their spleen tissues was removed for Flowcytometry analysis. The results of the research data analysis were processed using SPSS version 21.0 with a confidence interval (CI) of 95%.

Results and Discussion

To know the role of rIL-27, the IL-27 and TNFα expression was compared among groups, namely the normal group (no burn), burn group, and burn + rIL-27 group as indicated in table 1.

Table I. IL-27 and TNF-α expressions in the normal group (no burn), burn group, and burn + rIL-27 group (mean ± SD)

Variable	Normal (No Burn)		Burn		Burn + rIL-27	
	24-hour Observation (n=8)	P	24-hour Observation (n=8)	P	24-hour Observation (n=8)	P
IL-27 (mcg/ml)	1.352 ± 0.291	0.351*	1.625 ± 0.262	0.327*	1.912 ± 0.285	0.157*
TNF-α (mcg/ml)	0.996 ± 0.103	0.692*	1.342 ± 0.344	0.110*	0.975 ± 0.071	0.937*

Note: *The distribution of data analyzed using Shapiro-Wilk indicates that the data were normally distributed, P> 0.05; SD = Std deviation; mcg/ml = microgram/milliliters; n = number of subjects.

ANOVA analysis indicated that there were differences in each group (significance P<0.05) on IL-27 expression (P=0.03) and TNF-α expression (P=0.04). The analysis was continued using Post Hoc LSD (P<0.05) and the results indicated that there were significant differences in each group. To determine the relationship between IL-27 expression and TNF-α expression, Pearson analysis (r) = 0.912 was conducted at P=0.000 (significance at P<0.01 (2-tailed). Administration of rIL-27 significantly increased post-burn endogenous IL-27 expression (Fig.1). rIL-27 significantly decreased the post-burn TNF-α expression (Fig. 2).

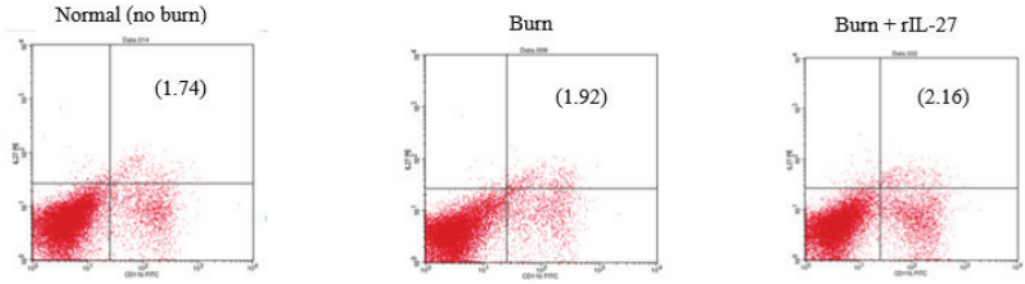

Figure 1. The effect of the burn injury on IL-27 expression in the macrophage cells (CD11b) spleen.

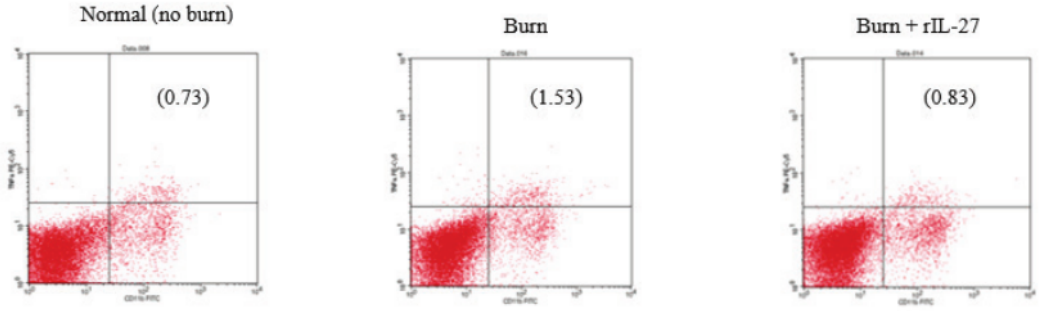

Figure 2. The effect of the burn injury on TNF-α expression in the macrophage cells (CD11b) spleen.

Burn injury induced the activation of TLRs on the surface of dendritic cells, further stimulating signaling pathways via the MyD88 protein adapter, TRAF6, and TAK1 then activates nuclear NF-κB and MAPKs thereby secreting the cytokine IL-27 into the circulation. IL-27 has two subunits, namely p28 and EBI3, while the IL27 receptors (WSX-1 and gp130) are expressed in macrophage cells[1]. WSX-1 and gp130 are transmembrane proteins that activate the Jak / STAT signaling pathway[8]. The most important negative regulator of gp130 / Jak / STAT signaling is SOCS3. The IL-27 receptor, gp130, is able to bind to SOCS3[9]. Diegelman's study, showed that IL-27 stimulated an increase in the expression of gp130 and SOCS3 (17.34 times compared to the control group) after 6 hours of IL-27 administration in ileal epithelial cells that had inflammatory bowel diseases (IBD)[10]. SOCS3 is a protein that is induced by many macrophage cells due to inflammation. SOCS3 is able to inhibit the activation of TRAF6 and TAK1 thus inhibiting the activation of NFkB to secrete proinflammatory cytokines such as TNF-α[11]. The excessive SOCS3 expression was proven to inhibit TNFR2 and STAT3 expressions from binding to the TNFR2 promoter and reduce TNF-α secretion in macrophages[12].

Conclusion

The administration of rIL-27 significantly increased endogenous IL-27, so that it decreased TNF-α after burns. The activated IL-27 cytokine bound to the gp130 receptor on the macrophage surface which regulates the macrophages to activate the JAK/STAT signaling pathway and induce SOCS3 which serves as an anti-inflammatory to suppress TNF-α secretion.

Ethical Clearance: Ethical Clearance of this study was obtained from the ethics commission of Veterinary Faculty, Airlangga University.

Conflict of Interest: There was no conflict of interests regarding the publication of this study.

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