

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 surfaces 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 TNF α 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 \pm 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 \pm 0.291	0.351*	1.625 \pm 0.262	0.327*	1.912 \pm 0.285	0.157*
TNF- α (mcg/ml)	0.996 \pm 0.103	0.692*	1.342 \pm 0.344	0.110*	0.975 \pm 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).

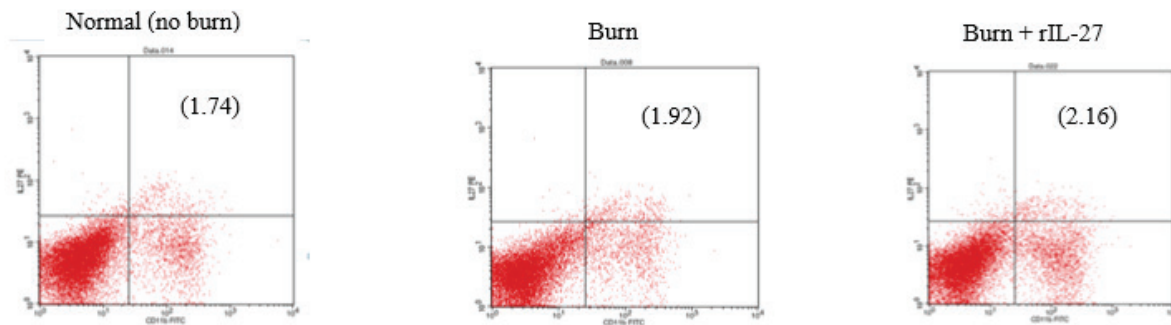


Figure 1. The effect of the burn injury on IL-27 expression in the macrophage cells (CD11b) spleen.

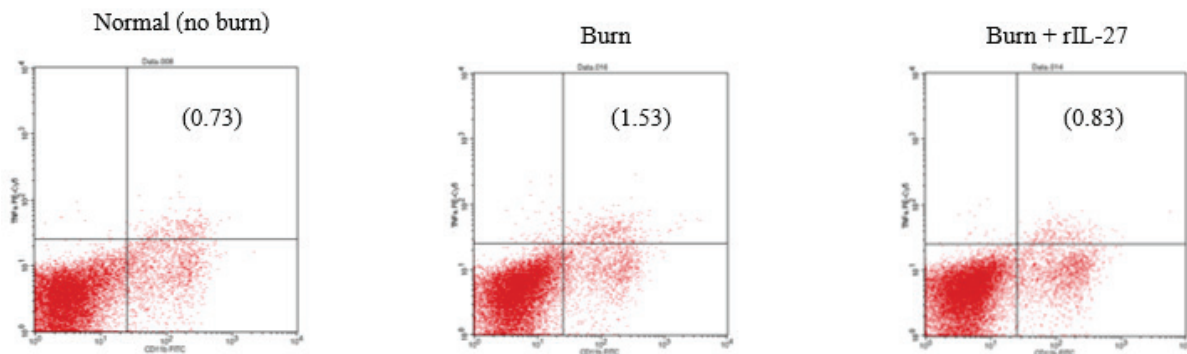


Figure 2. The effect of the burn injury on TNF- α expression in the macrophage cells (CD11b) spleen.

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- κ B 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 NF κ B 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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References

- Pflanz S, Hibbert L, Mattson J, Rosales R, Vaisberg E, Bazan JF, McClanahan TK, de Waal Malefyt R, Kastelein RA. WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27. *J Immunol* 2004; 172(4):2225–31.
- Yoshimura, H., Ohishi, M., Mori, H., Mukarami, M., Chinen, T., Aki, D., Hanada, T., Takeda, K., Akira, S., Hoshijima, M., Hirano, S., Chien, K. and Yoshimura, A. IL-6 induces an antiinflammatory response in the absence of SOCS3 in macrophages' *Nature Immunol* 2003; 4(6): 551–556.
- El Kasmi, K. C., Jeff Hols. Coffre, M., Mielke, L., de Pauw, A., Lhocine, N., Smith, A., Rutschman, R., Kaushal, D., Shen, Y., Suda, T., Donnelly, R., Myers, M., Alexander, W., Vignali, D., Watowich, S., Ernst, M., Hilton, D. and Murray, P. General nature of the STAT3-activated anti-inflammatory response. *J. Immunol* 2006; 177(11): 7880–7888
- Murray, P. The JAK–STAT signaling pathway: input and output integration. *J. Immunol* 2007; 178(5): 2623–2629.
- Wang, T., Niu, G., Kortylewski, M., Burdelya, L., Shain, K., Zhang, S., Bhattacharya, R., Bhattacharya, D., Heller, R., Coppola, D., Dalton, W., Jove, R., Pardoll, D., and Yu, H. Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. *Nature Med* 2004; 10(1): 48–54.
- Frobose, H., Sif, G., Peter, E., Heding, Heidi, M., Philip, C., Thomas, M. and Nils, N. Suppressor of cytokine signaling-3 inhibits interleukin-1 signaling by targeting the TRAF- 6/TAK1 complex. *Mol. Endocrino* 2006; 20(7): 1587–1596.
- Jeschke M., Gauglitz, G., Kulp, G., Finnerty, C., Williams, F., Kraft, R., Suman, O., Mlcak, R. and Herndon, D. Long-term persistence of the pathophysiologic response to severe burn injury. *PLoS One* 2006; 6(7): e21245.
- Villarino AV, Stumhofer JS, Christian .JM, Saris, R.A. Kastelein, F.J. de Sauvage, C.A. Hunter. IL-27 limits IL-2 production during Th1 differentiation, *J. Immunol* 2006;176(1) 237–247.
- Stumhofer JS, Laurence A, Wilson EH, Huang E, Tato CM, Jonson LM, Villarino AV, Huang Q, Yoshimura A, Sehy D, Saris CJ, O'Shea JJ, Hennighausen L, Ernst M, Hunter CA. Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system, *Nat. Immunol* 2006; 7(9)937–45.
- Diegelmann J, Olszak T, Goke B, Blumberg RS, Brand S. A novel role for interleukin-27 (IL-27) as mediator of intestinal epithelial barrier protection mediated via differential signal transducer and activator of transcription (STAT) protein signaling and induction of antibacterial and anti-inflammatory proteins. *J Biol Chem* 2012; 287(1):286-98.
- Niwa, Y., Kanda, H., Shikauchi, Y., Matsubara, K., Kitagawa, T., Yamamoto, J., Kubo, T. and Yoshikawa, H. Methylation silencing of SOCS-3 promotes cell growth and migration by enhancing JAK/STAT and FAK signalings in human hepatocellular carcinoma. *Oncogen* 2006;; 24(42): 6406–6417.
- Kershaw, J., Murphy, J., Liau, N., Varghese, L., Laktyushin, A., Whitlock, E., Lucet, I., Nicola, N. and Babon, J. SOCS3 binds specific receptor-JAK complexes to control cytokine signaling by direct kinase inhibition. *Nature Structural & Molecular Biology* 2013; 20(3): 469–476.