Enhancement of IL23-Independent IL17 Level on Intraperitoneal Injection of *Candida albicans* Cell Wall in Wistar Male Rat

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Abstract

IL17 is one of the most interesting cytokines studied recently because that cytokine involve on pathogenesis of wide range diseases. Production of this cytokine involve IL6, IL1β and IL23. Many evidence proved that there is an elevation of IL17 in peripheral blood in *C. albicans* infection. The aims of this study was to analyzed the effect of C.albicans cell wall injection intraperitoneally in level of IL17 and IL23. C.albicans cell wall isolated form *C. albicans* ATCC 10231 were administered intraperitoneally for 5 days in two series with 14 days interval on 3 group of wistar male rat. The doses of *C. albicans* cell wall were 0.8mg/kg bw/d, 1.6 and 3.2mg/kgbw/d. Control group were injected by aquadest pro injection. IL17 and IL23 blood level analyzed by ELISA technique 24 hours after last injection. There was a significant increase in the level of IL17 but not accompanied by IL23. The elevation of IL23-independet IL17 in the exposure of *C. albicans* cell wall supported the hypothesis that as respond of pathogen, elevation of IL 17 not necessarily preceded elevation of IL23. This hypothesis implicated that the IL23-independent IL17 likely have more protective effect than pathogenic effect as such in autoimmune diseases.

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Introduction

IL17 is an inflammatory cytokine produced by activated T cells. The IL17 gene is located on chromosome 6.[1] IL17 consists of 6 members, IL17A, B, C, D, E and F. IL17A is a disulfide-bound homodimer glycoprotein consisting of 155 amino acids with a molecular weight of 35 kDa.[2] IL17 is mainly produced by Th17 cells, although eventually IL17 cytokines are now known to be secreted by other cell types apart from CD8+ T cells, γδ T cells, natural killer T (NKT) cells, natural killer (NK) cells, monocytes, macrophages, dendritic cells (DC), microglia, neutrophils, eosinophil, astrocytes, and oligodendrocytes.[3]

Th17 cells have recently emerged as a third independent T cell subset which may play an essential role in protection against certain extracellular pathogens. However, Th17 cells with specificity for self antigens are highly pathogenic and lead to the development of inflammation and severe autoimmunity.[4,5] IL17A in humans are associated with pathology in numerous autoimmune and inflammatory conditions, such as rheumatoid arthritis (RA), multiple sclerosis (MS), psoriasis, Crohn's disease, systemic lupus erythematosus (SLE), asthma, Behçet's disease, and hyper IgE syndrome. The biologic functions of IL17 are consistent with the chronic and destructive nature of inflammation.[6] In oral health, IL17 have a significant role in pathogenesis of periodontal disease. IL17 is an important component of periodontal tissue inflammation, which facilitates a movement to a 'pathogenic' microbial community.[7]

Interleukin-23 (IL-23) is a pro-inflammatory cytokine composed of two subunits, p19 and p40. The p40 subunit is shared with IL-12. IL-23 and IL-12 have different receptors and different effects. Whereas IL-12 induces development of Th1 cells, which produce interferon-γ, IL-23 is involved in differentiation of

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Th17 cells in a pro-inflammatory context and especially in the presence of TGF-β and IL-6.[4]

Overwhelming data in both humans and mice reveal a clear and surprisingly specific role for IL17 in protection against the fungus C. albicans, a commensal microbial flora of the human oral cavity, gastrointestinal tract and reproductive mucosa.[8] The infection of *C. albicans* have increased recently as a result of the increase the prevalence of immunological deficiency diseases and the use of broad spectrum antibiotic. *C. albicans* is the most cause of infection of oral mucosa but can cause infection in all of part in oral cavity. *C. albicans* are associated with endodontic infection. *C. albicans* is the most commonly persistent pathogen and has a role in the failure of endodontic treatment.[9]

The role of IL17 in host defense against fungal primarily shown in 2004, whereas mice without IL17-receptor inoculated by *C. albicans* i.v. decreased life sustainability compared with WT mice. Some research revealed that mutation of gens linked in IL17's path in human lead to vulnerability toward Chronic Mucocutan Candidiasis/CMC. Individu with mutation of IL17RA, IL17RC, IL17F or signaling molecule specific of IL17 family, ACT1, susceptible to CMC.[10]

In this research, we analyzed the level of IL17 and IL23 in peripheral blood Wistar Male Rat after C albicans cell wall intraperitoneally injection.

Materials and Methods

Isolation of *C. albicans* cell wall

The method of isolation of *C. albicans* cell wall used was a combination of physical using ultrasound homogenizer, chemical and thermal which was adapted the technique from de Groot, 2004. *C. albicans* ATCC 10231 scraped from culture were washed with cold H20 and 10mM Tris-HCL, pH 7.5. The cell was resuspended in 10mM Tris-HCl, pH 7.5 and disintegrated Ultrasound Homogenizer (Cole Parmer 4710 series). To remove non-covalently bound proteins and intracellular contaminants washed with 1 M NaCl and extracted with 50mM Tris-HCL pH7.8 containing 2% SDS, 100mM NA-EDTA and 40m βMercapethanol, 5 minutes 100^{o}C, then washed 3 times with water and freeze-dried.[11]

Rat

Three month-old, 150-200 gr, Wistar male rat were purchased from Independent Animal Experiment Development, Sleman, Jogjakarta, Indonesia. Rat were kept in plastic cages (3 per cage) under pathogen-free conditions in a room at 28 ± 2.5 °C and 59% ± 5% relative humidity under a 12:12-h light–dark cycle. Rat were given free access to standard food and water throughout the experiments. The protocols were approved by the health research Ethical Clearance Commission Faculty of Dental Medicine Airlangga University Surabaya on October 29th 2017 with Certificate number 246/HRECC.FODM/X/2017.

C. albicans administration

Rat were divided into 3 group, 2 group treatment group and one as control group. Administration of *C. albicans* cell wall was carried out in a modified manner from Yoshikane's research, 2014.[12] Two treatment group were was injected intraperitoneally with 8 mg/KgBW/D (T1 group) and 16 mg/KgBW/D (T2 group) of *C. albicans* cell wall for 5 consecutive days every for 2 cycles with an interval of 23 days (Figure 1). Control group were injected by aquadest pro injection. Rat were euthanized 24 hours after last injection with 100 mg/kgBW ketamine i.m.

Figure 1. Schedule of Rats treatment, ↓: day of injection *C. albicans* cell wall intraperitonelly, ⇩ : Termination of Rats.

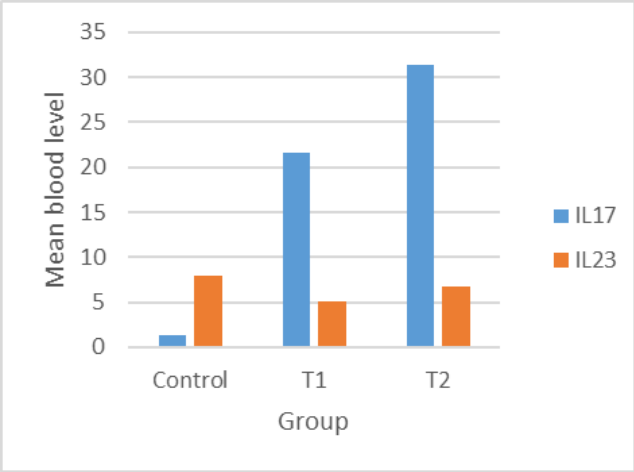
Pheriperal blood was collected from intracardial and put in a vacutainer containing EDTA. Blood plasma was separated to measure IL17 and IL23 levels by ELISA technique. Kit for ELISA which used were from Bioassay Technology Catalog No. E0115 Ra for measuring of IL17 levels and E0125Ra for measuring IL23 levels.

Statistical analysis

For determine the difference of the mean among group, the data was analyzed by Statistical Package for the Social Sciences (SPSS) 20.0 software for windows 10 by SPSS Inc., Chicago, United States. The significance was determined if $p < 0.05$.

Results

The mean blood level of IL17 in control group was 1.311 ± 1.016 pg/mL, in T1 group was 21.574 ± 10.865 pg/mL and in T2 group was 31.387 ± 8.490 pg/mL. IL23 mean blood level in control group was 7.965 ± 1.205 ng/mL, in T1 group was 5.038 ± 2,683 ng/mL and in T2 group was 6.742 ± 2.073 ng/mL. Mean of IL17 and IL23 blood level of all group illustrated in Graph 1.



Graph 1. Histogram level of IL17 and IL13.

The result of normality test and homogeneity test showed that all data were normal and homogeny. Result of multivariate and posthoc test in IL17 showed a significant difference among group, IL17 level control group was the lowest one, in T1 group, the level of IL17 was higher than control and the level of IL17 in T2 group was highest one ($p < 0.05$). Conversely, there is no significant differences in the level IL23 among all group.

Discussion

The results showed that exposure to *C. albicans* cell walls caused a significant increase in IL17 levels but this increase was not accompanied by an increase in IL23. The elevation of IL17 in animal model that exposure by *C. albicans* were proved in some research.[10, 13, 14] C albicans cell wall, one of virulence factor of C. albicans, are recognized by some PRRs, including Dectin 2. Signaling from Dectin-2 through Syk, PKCδ and CARD9-Bcl10-Malt1, induces several cytokines and chemokines including TNF, IL2, IL10, IL23, IL1β, IL6 and IL12. Recent research shows that the Dectin-2 signal involves phospholipase Cγ2 and mitogen-activated protein kinases (MAPKs) and selectively activates the c-Rel subunit NF-κB, through Malt1, encouraging cytokine production which promotes naïve polarization to Th17 ie IL23 and IL1β.[15]

In this study there was an increase in IL17 but not in IL23. This phenomenon is also found in some studies that proved the increase in IL17 could not depend on the presence of IL23. IL23-independent production of IL 17 was found in Il23r-Gfp homozygous knock-in mice and this interleukin have protective effect but not pathogenic effect during acute intestinal injury.[16] The other study prove that CD40 ligand–CD40 interactions were involved in the upregulation of IL-17A by lactic acid through IL-12/23p40 production. A new cytokine containing the IL-12/23p40 subunit, but not IL-23, IL-12 or the IL-12p40 homodimer, is a candidate for involvement in the up-regulation of IL-17A .[17] In study of Choroidal neovascularization (CNV), IL-17 had a strong potential for promoting neovascularization in a vascular endothelial growth factor–independent manner in laser-induced experimental CNV in mice. IL-23 was dispensable for IL-17 induction in the eye.[18]

The increased of IL17 level can influence of many systemic diseases. This study proves that *C. albicans* can increase levels of IL-17 independent IL-23 which has been shown to have no pathogenic effects. This result can affect the theory of the pathogenesis of the relationship between oral cavity diseases and systemic diseases. Some researchers have found that oral diseases is correlated with an increased risk of systemic disease, including diabetes mellitus and cardiovascular disease (CVD), as well as increased risk of heart attacks.[19]

Conclusion

This study shows an elevation of IL23-independent IL17 in Wistar Rat male after injection of *C. albicans* cell wall intraperitoneally

in two period of 5 consecutive days.

Conflicts of interest

The authors declare no conflict of interest.

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