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The Effect of IL-17A Kinoid Vaccine on Anti-dsDNA Levels and Plasma Cell Count in a Mouse Model of Systemic Lupus Erythematosus

Eka Tiar Rahmawati, Ermi Girsang
Magister Program of Biomedical Sciences, Faculty of Medicine, Dentistry and Health Sciences, Universitas Prima Indonesia, Medan, 20118, Indonesia
*E-mail : dr.eka.tr@gmail.com

ABSTRACT

Background: Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease characterized by dysregulated immune response, including overproduction of Interleukin-17A (IL-17A), which promotes B-cell differentiation into autoantibody-producing plasma cells. This study aimed to investigate the effect of an IL-17A kinoid vaccine on improving immune regulation in a Pristane-Induced Lupus (PIL) mouse model by measuring serum anti-double stranded DNA (anti-dsDNA) levels and splenic plasma cell count. Methods: A true experimental study with a randomized post-test-only controlled group design was conducted. Female Balb/c mice were divided into five groups: negative control (KN), positive control (KP, PIL-induced), and three treatment groups (P1, P2, P3) that were PIL-induced and received the IL-17A kinoid vaccine at doses of 125 µg/ml, 250 µg/ml, and 500 µg/ml, respectively, administered intramuscularly on days 0, 21, and 42. On day 60, serum anti-dsDNA levels were measured by ELISA, and splenic plasma cells (CD19+CD38+) were quantified by flow cytometry. Data were analyzed using One-Way ANOVA and Pearson correlation. Results: Induction with pristane successfully created a lupus model, evidenced by significantly positive ANA and proteinuria ($p<0.05$). The KP group showed a significant increase in plasma cell count (47.09 ± 3.77%) and anti-dsDNA levels (0.650 ± 0.01 µg/ml) compared to the KN group (27.71 ± 1.72%, p=0.000 and 0.517 ± 0.01 µg/ml, p=0.000, respectively). Treatment with the IL-17A kinoid vaccine significantly reduced both parameters. The most effective dose was 250 µg/ml (P2), resulting in a plasma cell count of 28.33 ± 1.70% (p=0.000 vs. KP) and anti-dsDNA level of 0.544 ± 0.16 µg/ml (p=0.000 vs. KP). A strong positive correlation was found between plasma cell count and anti-dsDNA levels (r=0.708, p=0.000). Conclusion: The IL-17A kinoid vaccine improves immune regulation in a PIL mouse model by significantly reducing the number of splenic plasma cells and the level of serum anti-dsDNA, with the most effective dose being 250 µg/ml..

Keywords: *Systemic Lupus Erythematosus, IL-17A, Kinoid Vaccine, Anti-dsDNA, Plasma Cells.*

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a complex, chronic autoimmune disease characterized by systemic inflammation and multi-organ damage due to a loss of self-tolerance and production of autoantibodies (Gurevitz et al., 2013). The global incidence of SLE is estimated at 5 million people, with significant morbidity and mortality, particularly in developing countries like Indonesia (Kalim et al., 2012). A key immunological feature of SLE

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is the production of autoantibodies against nuclear antigens, especially anti-double stranded DNA (anti-dsDNA), which plays a direct pathogenic role in tissue injury, such as lupus nephritis (Yung & Chan, 2015; Zhang et al., 2016).

These autoantibodies are produced by autoreactive plasma cells, the terminal differentiation stage of B cells. The number of plasma cells in the periphery correlates with disease activity and anti-dsDNA levels in SLE patients (Liu et al., 2011). The differentiation and survival of these cells are influenced by various cytokines, including Interleukin-17A (IL-17A). IL-17A, primarily produced by T-helper 17 (Th17) cells, is a pro-inflammatory cytokine elevated in SLE patients and correlates with disease severity, including renal involvement (Wong et al., 2008; Nalbandian et al., 2009). IL-17A synergizes with B-cell activating factor (BAFF) to promote B-cell activation, differentiation into plasma cells, and subsequent autoantibody production (Doreau et al., 2009).

Current standard therapies for SLE, such as corticosteroids and immunosuppressants, often have limited efficacy and significant side effects. Biologic agents are promising but costly. Therefore, developing novel, effective, and accessible therapies is crucial. Kinoid vaccination represents an innovative approach. A kinoid vaccine consists of a cytokine derivative, in this case, IL-17A, that has been rendered biologically inactive but remains immunogenic. Upon administration, it induces neutralizing antibodies against the target cytokine, thereby reducing its pathological overproduction (Bizzini et al., 2010). This study aims to evaluate the efficacy of a novel IL-17A kinoid vaccine in improving immune regulation by reducing splenic plasma cell count and serum anti-dsDNA levels in a Pristane-Induced Lupus (PIL) mouse model.

METHODS

Study Design and Animals

This was a true experimental study using a randomized post-test-only controlled group design. The study protocol was approved by the institutional ethical committee. A total of 26 female Balb/c mice (aged 6-8 weeks, weighing 25-30 g) were used. After one week of acclimatization, the mice were divided into two initial groups: Group 1 (n=7, healthy controls) and Group 2 (n=19, PIL model). The PIL model was established via a single intraperitoneal injection of 0.5 mL pristane (Sigma-Aldrich, USA). At week 8, successful induction was confirmed by positive Anti-Nuclear Antibody (ANA) and proteinuria tests in 3 mice from each group. The remaining mice were then reallocated into five final groups (n=4 each):

- KN: Negative Control (healthy, no treatment)
- KP: Positive Control (PIL-induced, no vaccine)
- P1: PIL-induced + IL-17A kinoid vaccine 125 µg/ml
- P2: PIL-induced + IL-17A kinoid vaccine 250 µg/ml
- P3: PIL-induced + IL-17A kinoid vaccine 500 µg/ml

IL-17A Kinoid Vaccine Preparation and Administration

The IL-17A kinoid vaccine was prepared by conjugating recombinant mouse IL-17A (BioLegend, USA) with Keyhole Limpet Hemocyanin (KLH; Sigma-Aldrich, USA) as a

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carrier protein using the aldehyde method as described by Zagury et al. (2009). The conjugate was mixed 1:1 (v/v) with Complete Freund's Adjuvant (CFA) for the first injection and Incomplete Freund's Adjuvant (IFA) for booster injections. The vaccine was administered intramuscularly in a 0.2 mL volume on days 0, 21, and 42.

Sample Collection and Measurements

On day 60 after the first vaccination, all mice were sacrificed. Blood was collected via cardiac puncture for serum separation. Spleens were harvested for plasma cell analysis.

- **Anti-dsDNA Measurement:** Serum anti-dsDNA levels were quantified using a commercial sandwich ELISA kit (BioLegend, USA) according to the manufacturer's instructions. Results are reported in µg/ml.
- **Plasma Cell Count:** Splenic mononuclear cells were isolated. Plasma cells were identified as CD19+ and CD38+ double-positive cells by flow cytometry (BD FACS) using PE anti-mouse CD19 and PerCP anti-mouse CD38 antibodies (BioLegend, USA). The results are expressed as a percentage of 1x10^6 analyzed cells.

Statistical Analysis

Data were analyzed using SPSS software. Normality and homogeneity of variance were tested using the Kolmogorov-Smirnov test. One-Way ANOVA was used for intergroup comparisons, followed by the Tukey HSD post-hoc test. The Pearson correlation test was used to analyze the relationship between plasma cell count and anti-dsDNA levels. A p-value of <0.05 was considered statistically significant.

RESULTS

Confirmation of Lupus Model

Induction with pristane for 8 weeks successfully created the lupus model. The PIL-induced group (Grup 2) showed significantly higher levels of ANA (87.64 ± 12.23 µg/ml vs. 21.01 ± 12.94 µg/ml, p=0.003) and proteinuria (65.8 ± 13.86 mg/dl vs. 10.8 ± 0.90 mg/dl, p=0.002) compared to the healthy control group (Grup 1).

Effect of IL-17A Kinoid Vaccine on Plasma Cell Count

Flow cytometry analysis showed that the plasma cell count in the spleen was significantly higher in the KP group (47.09 ± 3.77%) compared to the KN group (27.71 ± 1.72%, p=0.000). Administration of the IL-17A kinoid vaccine significantly reduced the plasma cell count in all treatment groups compared to the KP group. The most effective reduction was observed in the P2 group (250 µg/ml), which showed a count (28.33 ± 1.70%) comparable to the healthy control (KN). The P3 group (500 µg/ml) showed a less pronounced reduction (30.09 ± 2.58%) compared to P2 (Table 1, Figure 1).

Table 1. Effect of IL-17A Kinoid Vaccine on Plasma Cell Count and Anti-dsDNA Level

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Group	Dose (µg/ml)	Plasma Cell Count (%CD19+CD38+, Mean ± SD)	p-value (vs. KP)	Anti-dsDNA Level (µg/ml, Mean ± SD)	p-value (vs. KP)
KN	-	27.71 ± 1.72	0.000*	0.517 ± 0.01	0.000*
KP	-	47.09 ± 3.77	0.000*	0.650 ± 0.01	0.000*
P1	125	41.62 ± 2.16	0.050*	0.597 ± 0.02	0.005*
P2	250	28.33 ± 1.70	0.000*	0.544 ± 0.16	0.000*
P3	500	30.09 ± 2.58	0.000*	0.611 ± 0.01	

*Note: Statistically significant ($p < 0.05$); KN: Negative Control; KP: Positive Control; P: Treatment Group.

Effect of IL-17A Kinoid Vaccine on Anti-dsDNA Level

Similarly, serum anti-dsDNA levels were significantly elevated in the KP group (0.650 ± 0.01 µg/ml) compared to the KN group (0.517 ± 0.01 µg/ml, p=0.000). Treatment with the IL-17A kinoid vaccine significantly lowered anti-dsDNA levels in all treatment groups. The P2 group (250 µg/ml) again showed the most substantial decrease (0.544 ± 0.16 µg/ml), while the P1 and P3 groups showed more modest reductions (Table 1, Figure 1).

Correlation between Plasma Cell Count and Anti-dsDNA Level

A Pearson correlation analysis revealed a strong positive correlation between the number of splenic plasma cells and the serum level of anti-dsDNA ($r = 0.708$, $p = 0.000$).

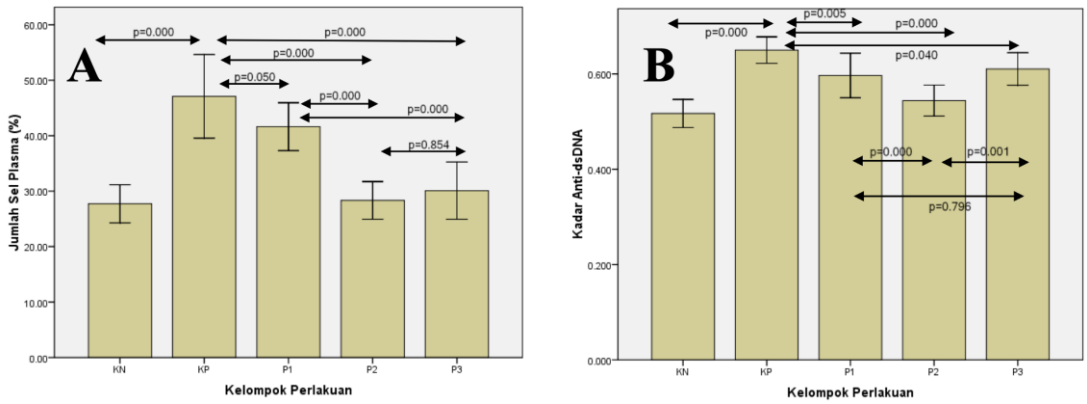

Figure 1. Graphical representation of the quantitative data from Table 1, showing the reduction in (A) plasma cell count and (B) anti-dsDNA levels across treatment groups, with the most significant effect at the 250 µg/ml dose (P2). *$p < 0.05$ vs. KP; # $p < 0.05$ P2 vs. P3.

DISCUSSION

This study demonstrates that active immunization with an IL-17A kinoid vaccine can ameliorate key immunological abnormalities in a PIL mouse model of SLE. The PIL model was successfully established, as confirmed by the significant increase in ANA and proteinuria, consistent with previous studies (Reeves et al., 2009; Chowdhary et al., 2007).

The central finding is that the IL-17A kinoid vaccine significantly reduced both the number of autoreactive plasma cells in the spleen and the concentration of pathogenic anti-dsDNA

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antibodies in the serum. The efficacy was dose-dependent up to a point, with the 250 µg/ml dose (P2) proving most effective. The reduced plasma cell count aligns with the known role of IL-17A in promoting B-cell biology. IL-17A acts synergistically with BAFF to enhance B-cell survival, proliferation, and differentiation into antibody-producing plasma cells (Doreau et al., 2009). By inducing neutralizing antibodies against IL-17A, the kinoid vaccine disrupts this synergistic signal, thereby curbing the generation of autoreactive plasma cells. This mechanism is supported by studies showing that IL-17 blockade can reduce germinal center size and B-cell responses (Mitsdoerffer et al., 2010).

The concomitant decrease in serum anti-dsDNA levels is a direct consequence of the reduced plasma cell population, as these cells are the primary source of this pathogenic autoantibody. The strong positive correlation (r=0.708) between plasma cell count and anti-dsDNA level further solidifies this causal relationship. This finding is clinically relevant, as anti-dsDNA is a key driver of tissue injury, particularly in lupus nephritis, through immune complex deposition and molecular mimicry (Wang et al., 2014; Yung & Chan, 2015).

An interesting observation was the diminished effect at the highest dose (500 µg/ml, P3). This phenomenon may be attributed to "high-dose tolerance" or "high-dose suppression," where excessive antigen exposure can lead to T-cell anergy or activation-induced cell death (AICD), thereby blunting the helper T-cell response necessary for effective B-cell activation and antibody production against the kinoid (Michallet et al., 2004). This underscores the importance of dose optimization in vaccine development.

While promising, this study has limitations. The role of BAFF, a critical partner for IL-17A, was not investigated. The long-term protective efficacy and potential risk of infections due to sustained IL-17A neutralization—a cytokine important for mucosal immunity against fungi and extracellular bacteria—require further evaluation (Ronnblom & Elkon, 2010). Future studies should include a broader dose range and confirm conjugate formation using methods like Western blot.

CONCLUSION

The IL-17A kinoid vaccine effectively improves immune regulation in a Pristane-Induced Lupus mouse model. It significantly reduces the number of splenic plasma cells and the level of serum anti-dsDNA, with an optimum dose of 250 µg/ml. These findings highlight the potential of anti-cytokine kinoid vaccination as a novel therapeutic strategy for SLE, warranting further investigation into its long-term efficacy and safety.

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