

**Original Research Report****SEROLOGICAL PROFILES OF SYSTEMIC LUPUS ERYTHEMATOSUS IN HUMANIZED MICE AND PRISTANE-INDUCED LUPUS MODELS**Syahrul Chilmi<sup>1</sup> , Dimas Ikhsan Airlangga<sup>2\*</sup> , Hani Susianti<sup>1</sup> , Kusworini Handono<sup>1</sup> <sup>1</sup>Department of Clinical Pathology, Faculty of Medicine, Universitas Brawijaya; Saiful Anwar Regional General Hospital, Malang, Indonesia<sup>2</sup>Master's Program in Biomedical Science, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia**ABSTRACT**

Systemic lupus erythematosus (SLE) therapy requires further study because of the current lack of treatment efficacy. However, due to ethical restrictions, researchers use experimental animals as a substitute for human studies. Commonly used models for studying lupus include the pristane-induced mouse model and the recently developed humanized mouse model. In the latter model, human immune cells are transplanted into immunodeficient mice. This study compared the serologic profiles of lupus antibodies, namely antinuclear antibodies (ANA) and anti-double stranded DNA (anti-dsDNA), in both mouse models to determine their efficacy as lupus animal models. Thirty BALB/c mice (*Mus musculus*) were used as subjects and divided into three groups: K1, K2, and K3. K1 served as the control group, consisting of healthy mice that received a placebo. The K2 mice were intraperitoneally injected with 0.5 cc of pristane. The K3 mice were transplanted with stem cell cultures from SLE patients, creating humanized mice with immune deficiencies. The mice were observed for 16 weeks, during which the ANA and anti-dsDNA levels in their serum were obtained for analysis using the Kruskal-Wallis test ( $p < 0.05$ ). The comparison revealed differences in the average ANA and anti-dsDNA levels among the three groups. K3 had the highest ANA and anti-dsDNA levels, followed by K1 and K2. The Kruskal-Wallis test indicated no significant differences in the mean levels of ANA ( $p = 0.156$ ) and anti-dsDNA ( $p = 0.061$ ). In conclusion, the humanized mouse model showed higher ANA and anti-dsDNA antibody levels compared to the pristane-induced mouse model, although the differences were not statistically significant. This suggested that the humanized mouse model of lupus is a promising tool for studying the disease and testing potential therapeutic interventions.

**Keywords:** Systemic lupus erythematosus; humanized mouse model of lupus; pristane-induced lupus model; disease**\*Correspondence:** Dimas Ikhsan Airlangga, Master's Program in Biomedical Science, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia. Email: [dimasikhsanairlangga10@gmail.com](mailto:dimasikhsanairlangga10@gmail.com)**Article history**

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**Highlights:**

1. This study compared the serological markers of pristane-induced mice to humanized mouse models of lupus achieved by transplanting stem cells from lupus patients, which is a novel method in Indonesia.
2. This study will allow for more accurate research into the pathophysiology of the disease and the development of new lupus treatment strategies.

**INTRODUCTION**

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that affects multiple organ systems. It is characterized by the abnormal activation of immune cells and the dysregulation of cytokine secretion, antibody production, and immune complex formation (Tang et al. 2021, Justiz Vaillant et al. 2024). Lupus is more prevalent in young women and women of childbearing age than

in men. The disease is one of the leading causes of death among young women in the United States. Mortality rates among SLE patients have decreased in recent decades. However, the mortality rate of SLE patients remains twice as high as that of patients without SLE (Ocampo-Piraquive et al. 2018).

The pathophysiology of SLE disease involves an impaired or loss of immune tolerance in genetically susceptible individuals who are exposed to

environmental factors, resulting in the activation of autoimmune responses. This leads to an increased activity of cytokine release, complement activation, and autoantibody production, which ultimately contributes to functional damage and cell apoptosis in various organ systems, especially the kidney and cardiovascular systems (Tayem et al. 2022, Scheen et al. 2022). According to Tang et al. (2021), conventional therapy for SLE focuses on the administration of drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, cyclophosphamide (CTX), azathioprine, and methotrexate. It is noteworthy that while these drugs provide curative effects in some SLE patients, they fail in other SLE patients due to severe side effects or incompatibility with conventional immunosuppressive treatment. Additionally, there are patients who do not respond adequately to the combined therapy of steroids and immunosuppressants, which is further coupled with the many unwanted side effects linked to this type of therapy (Wang et al. 2015).

Research on SLE therapy is a field that continues to be widely developed, as no recent studies have demonstrated a high level of effectiveness in the treatment of SLE over the past twenty years. Researchers are more inclined to use experimental animals due to ethical limitations in clinical trials involving human subjects. The frequently used spontaneous lupus models are NZB/NZW F1 (BW) mice and MRL/lpr mice. These mice can exhibit clinical and serologic features of lupus, such as antinuclear antibodies (ANA) and anti-double-stranded DNA (anti-dsDNA) (Li et al. 2017, Tejon et al. 2020). However, obtaining these animal models in Indonesia is costly and difficult. Another commonly used mouse model is the pristane-induced lupus (PIL) model. In this model, mice are injected with pristane to develop lupus-like symptoms characterized by autoantibody production, immune complex depletion, inflammation, and various clinical manifestations such as arthritis and hair loss (alopecia) (Zschaler et al. 2014). Although this model is useful for studies on the mechanism of lupus, it cannot fully describe the complexity of SLE pathogenesis in humans. Therefore, the results of the studies cannot be directly applied to patients (Pittenger et al. 2019, Adigbli et al. 2020).

Mouse models with human-like features are necessary to observe human cells within mice in vivo. Humanized mouse models of lupus are made by reconstructing human cells in mice. This can be achieved by transferring either peripheral blood mononuclear cells from SLE patients to mice lacking a functional immune system or by introducing human hematopoietic stem cells into immunodeficient mice, followed by injecting

pristane into the peritoneal cavity (Gunawan et al. 2017, Mihaylova et al. 2020, Chen et al. 2022). The models are expected to produce autoantibodies and other immune system abnormalities that mimic those in SLE patients, allowing for more accurate research into the pathophysiology of the disease and the development of new treatment strategies. Therefore, this study aimed to compare the differences in clinical and serological signs of SLE between the humanized mouse model group and the pristane-induced model group.

## MATERIALS AND METHODS

The Health Research Ethics Committee of Dr. Saiful Anwar Regional General Hospital, Malang, Indonesia, issued the ethical approval for this study on 23/12/2023 under protocol No. 400/281/K.3/302/2023. This study was conducted in the Laboratory for Experimental Animal Development (*Laboratorium Pengembangan Hewan Coba*) and Central Biomedical Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia, from June to December 2023. This study used a pure experimental research design, specifically utilizing a posttest-only controlled group design. The research was carried out in vivo using female BALB/c mice (n=30) to develop humanized mouse models of lupus and pristane-induced lupus models (Gridley & Murray 2022). The grouping was performed randomly using a simple random sampling method. The mice were divided into K1, K2, and K3 groups, with each group consisting of 10 mice. The control group (K1) comprised healthy mice that received a placebo in the form of a 0.5 cc-intraperitoneal injection of normal saline. The K2 group received a 0.5-mL intraperitoneal injection of pristane. The K3 group consisted of humanized mouse models of lupus that were made immunodeficient and received cell transplants from SLE patients (Justice & Dhillon 2016).

The materials used in this study included standard feed, pristane (Santa Cruz, USA), cyclophosphamide, hematopoietic stem cell culture, Mouse ANA and anti-dsDNA enzyme-linked immunosorbent assay (ELISA) kits, mice cage, GWXJ80 cobalt radiotherapy device, 1 cc syringe, micropipette, falcon tube, glass tube, 37 °C incubator, and microplate reader. During the experimental animal preparation, the BALB/c mice were initially acclimatized for seven days. They were then provided with standard feed and placed in a cage with a husk mat that was cleaned every three days. In the preparation of pristane-induced lupus models, healthy mice aged 6–8 weeks were intraperitoneally injected with 0.5 mL of pristane (Santa Cruz, USA), then waited for 6 weeks to self-

develop the lupus antibodies. The preparation of the humanized mouse models started by obtaining hematopoietic stem cells from SLE patients and subsequently culturing them (Chen et al. 2022). In the meantime, mice aged 6–8 weeks were made immunodeficient by intraperitoneally injecting 80 mg/kg bw of cyclophosphamide (CPA) twice with an interval of two weeks. In week 8, the mice were exposed to Co-60 gamma radiation at a dose of 6 Gy twice, with an interval of four hours. After 24 hours of radiation administration,  $5 \times 10^6$  cells from the hematopoietic stem cell culture were injected into the femur bone marrow of the mice while they were under anesthesia by isoflurane inhalation. The mice were then observed for changes in body weight and general condition until week 16 (Figure 1).

The evaluation of SLE serologic markers began at week 16. The special laboratory markers (i.e., ANA and anti-dsDNA) from mouse serum were examined using the ELISA method. The Mouse ANA and anti-dsDNA ELISA kits were utilized to detect antibodies in the mice according to the two markers (González et al. 2015). The ELISA procedures, in general, were conducted in several steps. The coating antigen was dissolved at a ratio of 1:20 in an ELISA plate and then incubated for one night at 40 °C. After incubation, it was washed using phosphate-buffered saline with Tween® (PBS-T), then blocked with 1% bovine serum albumin (BSA), and washed again using PBS-T. The primary antibody (serum) was diluted at a ratio of 1:500 in phosphate-buffered saline (PBS). Afterward, 100 µL of the diluted solution was added to the tubes and incubated for one hour. The tubes were washed three times using 300 µL of 0.2% PBS-T. After incubating for one hour, the enzyme-labeled secondary antibodies, i.e., anti-Mouse Immunoglobulin G (IgG) and anti-human IgG, were added to the tubes at a ratio of 1:1000. The tubes were washed with 0.2% PBS-T, then streptavidin conjugated with horseradish peroxidase (SA-HRP) was added at a ratio of 1:1000, followed by additional incubation for one hour. Subsequently, the cells were washed with 0.2% PBS-T before the substrate SureBlue 3,5,3',5'-tetramethylbenzidine (TMB) was added and incubated for 30 minutes. Without removing the SureBlue TMB, the reaction was stopped by adding 1N hydrochloric acid (HCl), and the mixture was then incubated for 15 minutes. Readings were collected using an ELISA reader ( $\lambda=450$  nm). The levels of ANA and anti-dsDNA were measured and reported in IU/mL (Rekvi 2014).

The data analysis was performed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, N.Y., USA). The Shapiro-Wilk normality test and the homogeneity of variance test were performed as prerequisites for parametric tests. Comparative tests between the parameters were

performed by one-way analysis of variance (ANOVA) when the data fulfilled the criteria of normality and homogeneity. If the data did not meet these criteria, the Kruskal-Wallis test was used instead. The confidence level for these tests was set at 95%, and a value of  $p < 0.05$  was deemed significant (Cleophas & Zwinderman 2016).

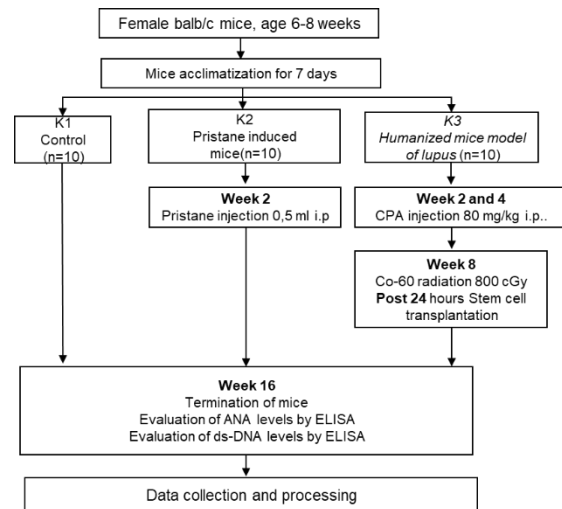


Figure 1. The diagram of the study timeline and procedures.

## RESULTS

The results of the antibody examination using the ELISA method indicated the levels of ANA in the serum of mice across the study groups. In the control group (K1), the mean ANA level along with the standard error (SE) for the mean value was  $9.192 \pm 0.622$  pg/mL. The mean ANA level was  $13.661 \pm 3.466$  pg/mL in the group of pristane-induced lupus models (K2). Lastly, the mean ANA level was  $16.944 \pm 3.726$  pg/mL in the group of humanized mice (K3). Figure 2 illustrates the mean levels of ANA across the three groups.

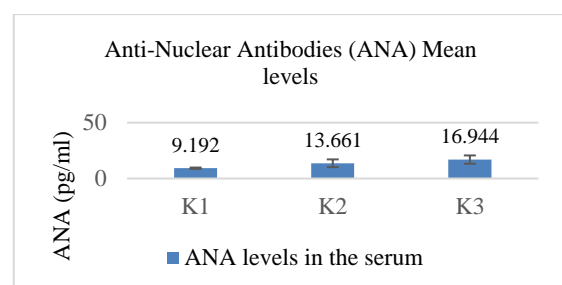


Figure 2. Mean levels of ANA in the serum after intervention.

The results of the Shapiro-Wilk normality test showed that the data were not evenly distributed

( $p < 0.05$ ). Therefore, the data were further analyzed using the Kruskal-Wallis test, which resulted in a value of  $p = 0.156$ . The value indicated that there was no statistically significant difference between each group. However, it should be noted that the ANA levels in the group of humanized mice (K3) were higher than those in the group of pristane-induced models (K2).

Anti-dsDNA antibody levels were the other parameter examined in this study, as shown in Figure 3. The mean anti-dsDNA antibody levels were  $61.380 \pm 4.806$  pg/mL in the K1 group,  $129.780 \pm 42.631$  pg/mL in the K2 group, and  $224.998 \pm 71.070$  pg/mL in the K3 group. The humanized mice in the K3 group had the highest anti-dsDNA levels compared to the control group (K1) and the group of pristane-induced mice (K2). These results were similar to those obtained from the ANA levels examination.

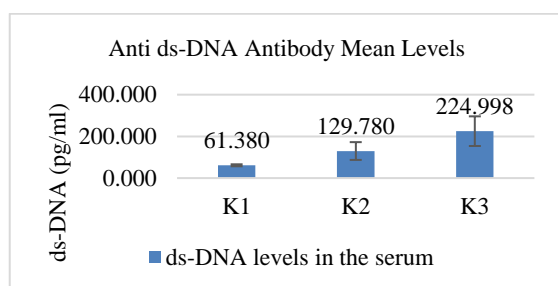


Figure 3. Post-intervention mean levels of anti-dsDNA in the mouse serum.

The Shapiro-Wilk normality test revealed a non-normal distribution of the data on anti-dsDNA levels ( $p < 0.05$ ). Consequently, the analysis proceeded with the Kruskal-Wallis test, yielding a value of  $p = 0.061$ . There was no statistically significant difference observed between all the groups. Although the difference was not statistically significant, there was a pattern indicating that the group of humanized mice (K3) successfully developed higher levels of lupus antibodies compared to the group of pristane-induced models (K2).

## DISCUSSION

Our study has demonstrated a notable disparity in antibody production between the humanized mice and the pristane-induced mice. The observed phenomenon underscores the effectiveness of the humanized mouse model in generating a robust immune response, surpassing that of the pristane-induced model. Several factors are likely responsible for this notable contrast, indicating the need for further exploration. However, we must firstly understand the underlying pathophysiology of

SLE in both models. In the pristane-induced model, the mice received intraperitoneal administration of pristane oil. This induced irritation in the peritoneum, thereby increasing the production of monoclonal antibodies from ascites upon hybridoma injection. This process ultimately led to the onset of autoimmunity. The activation of polyclonal B cells triggered by pristane injection can result in the generation of autoantibodies and the onset of autoimmune conditions (Richard & Gilkeson 2018, Jang et al. 2021). Furthermore, immunological changes, such as the development of SLE in mice following pristane injection, are associated with alterations in the immune response, including shifts in the expression of activated and inhibited Fc receptors. The findings of this study are consistent with those of a prior study conducted by da Costa et al. (2019). Their experiment has successfully established a murine model of lupus in female BALB/c mice by administering a single intraperitoneal injection of 0.5 mL of pristane. This resulted in the appearance of specific anti-dsDNA autoantibodies for SLE in the sera of BALB/c mice.

The development of humanized mice involves transplanting or engrafting human cells into immunocompromised mice, which allows the animals to produce cells with human-like characteristics. This approach enables researchers to study aspects of the human immune system and stem cells in vivo (Pittenger et al. 2019). In general, there are currently two primary methods utilized for creating humanized mouse models of lupus. The first method entails transferring human peripheral blood mononuclear cells (PBMCs) or peripheral blood lymphocytes (PBLs) from SLE patients into immunodeficient mice (Gunawan et al. 2017, Chen et al. 2022). In the PBMC approach, cells obtained from the patient's blood are introduced into immunodeficient mice via either intravenous (IV) or intraperitoneal (IP) injection. Conversely, in the second method known as the hematopoietic stem cell (HSC) method, blood stem cells are administered intravenously into immunodeficient mice, followed by intraperitoneal administration of pristane. The pristane administration in the HSC technique is intended to induce SLE in mice, eliciting the production of human ANA and thereby instigating a lupus-like phenotype in the animal models (Chen et al. 2022).

In this study, we tried to transplant hematopoietic stem cells from lupus patients directly into immunodeficiency mice to observe their cell growth and assess their ability to trigger signs of SLE, such as the appearance and increase of ANA and anti-dsDNA antibodies. Hematopoietic stem cells possess the capacity for specific and extensive differentiation into various cell lineages, leading to the production of blood cells and mature immune

cells in humans (Lee & Hong 2020). When transplanted into mice, these cells are anticipated to retain their human characteristics and influence the development of immune cells within the host. Stem cells derived from individuals with lupus display altered functionality and properties compared to healthy stem cells (Grigoriou et al. 2020). It can be inferred that when stem cells from lupus patients are introduced into mice, they also carry the distinctive traits of lupus-afflicted human cells. Over a short duration, these transplanted stem cells undergo further differentiation into diverse myeloid and lymphoid immune cells, exhibiting characteristics of human lupus, including immune system dysregulation and autoantibody production. The results of this study showed that this method was able to increase lupus antibodies even more than the pristane-induced lupus model.

According to Tu & Zheng (2016), humanized mice embody a more physiologically relevant system for studying immune responses due to the presence of human cells. By transplanting human immune cells into immunocompromised mice, we effectively recreated a microenvironment conducive to human-like immune reactions. In contrast, the pristane-induced lupus models relied solely on murine immune components, potentially limiting their capacity to accurately mimic human immune responses. The intrinsic differences in immune cell populations between the two models could account for the observed variations in antibody production (Freitas et al. 2017). Humanized mice harbor a repertoire of human immune cells, including T cells, B cells, and antigen-presenting cells, which collectively contribute to a diverse and dynamic immune response. Conversely, pristane-induced lupus models may not fully mirror the complexity and functionality of the human immune system (Gunawan et al. 2017, Chen et al. 2022).

The superior antibody production in humanized mice might be attributed to the enhanced compatibility between human immune cells and the murine host environment. The immunodeficient background of the recipient mice provides a permissive niche for engraftment and proliferation of human cells, fostering the development of functional human immune responses (Mian et al. 2021). On the other hand, the pristane-induced lupus models might impose additional stressors or limitations on murine immune cells, potentially compromising their ability to mount robust antibody responses. The increased production of ANA and anti-dsDNA antibodies in humanized mouse models might correlate with the severity of lupus-like symptoms observed. Elevated antibody levels are often associated with more pronounced immune-mediated tissue damage and systemic manifestations of lupus, such as nephritis and arthritis (Pan et al.

2014, Pisetsky & Lipsky 2020, Cai et al. 2022). As the elevated antibody levels in humanized mouse models may reflect a worsening disease phenotype, this model can be useful for studying disease progression.

### Strength and limitations

In this study, the levels of ANA and anti-dsDNA antibodies in humanized mouse models did not show statistically significant differences compared to the pristane-induced lupus models. This implies that although there might be differences in absolute antibody concentrations, such differences lacked statistical significance. Therefore, it is important to consider alternative explanations and potential implications, notably the limitations of the model design and the need for more sensitive measurements. Our results do emphasize the benefit of the humanized mouse models compared to the pristane-induced lupus models for investigating immune responses and antibody production. Future studies should put a high priority on understanding the complex interactions between human immune cells and the murine host environment. This is crucial for improving the capabilities of humanized mouse models and enhancing therapeutic strategies against SLE.

### CONCLUSION

The humanized mouse model of lupus exhibited higher antibody production than the pristane-induced lupus model. This can be attributed to its closer resemblance to human immune system characteristics. The humanized mouse model offers enhanced translational relevance and aids in advancing our understanding of lupus pathology and potential therapeutic interventions.

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### Conflict of interest

None.

### Ethical consideration

The ethical approval for this study was issued by the Health Research Ethics Committee of Dr. Saiful Anwar Regional General Hospital, Malang, Indonesia, under protocol No. 400/281/K.3/302/2023 dated 23/12/2023.

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## Author contribution

SC carried out the conception, design, and experimentation, as well as provided study materials, obtained the funding, and collected and assembled the data. DIA drafted the article as well as contributed to the experimentation and the collection, analysis, and interpretation of the data. HS contributed to the conception and design, obtainment of funding, experimentation, collection and assembly of the data, and critical revision of the article for important intellectual content. KH contributed to the conception and design, experimentation, and critical revision of the article for important intellectual content. All authors participated in the final approval of the article for publication.

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