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Pristane modulates specific changes on T cell dependent pathway of lupus in non-F1 BALB/c mice

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ABSTRACT

Animal modeling for lupus is a crucial step in the process of discovering efficacious drugs. There are many drug candidates that have potential benefits for the treatment of lupus, but finding an appropriate model remains challenging. The appropriate model based on the literature is an induction model that uses 2,6,10,14 tetramethylpentadecane (TMPD) in female Balb/c mice. The TMPD increases the probability of damage beyond lupus, to the joint and kidneys. Therefore, the purpose of this study was to develop a lupus model by TMPD to test a drug candidate. The experimental method was measuring many biomarkers involved in the TMPD mechanism to obtain lupus-like disease mice. We measured $CD4^+CD25^+FOXP3^+$ and $CD4^+CD62L^+$ T-regs, $CD123^+IFN\text{-}\alpha^+$ dendritic cells (flow cytometry); total leukocytes (Turk staining); anti-Sm antibody (ELISA); and renal and joint histology (HE staining). After the 6th month, there were reduction ($p<0.05$) of the T regulatory percentage of $CD4^+CD25L^+$ T cells (Naïve=19.39±3.06%, TMPD-treated=5.72±3.43%) and $CD25^+FOXP3^+$ T cells (Naïve=10.32±4.47%, TMPD-treated=7.70±4.47%), meanwhile, the IFN-α increased significantly ($p<0.05$), (Naïve=6.92±8.67%, TMPD-treated=11.42±0.95%), and the total leukocytes increased ($p<0.05$) (Naïve=9,800±1,698, TMPD-treated=17,500±1,490 cells/mm^3). The anti-Sm antibody was also present in the TMPD-treated mice as one cause which led to renal and joint structural disorders. The biomarkers were analyzed by using Independent T-test, so this positive lupus model with its tested biomarkers is valid and can be used to test drugs for lupus.

Keywords: Lupus Animal, Tetramethylpentadecane, IFN-α, Lupus Biomarkers, T Regulatory

INTRODUCTION

Lupus is an autoimmune disease with various severe signs. Lupus manifestations occur by many triggering factors, such as UV light from sunshine so that women in tropical countries have the higher risk than others. The light has a power to increase the activity of auto-reactive T cells in lupus patients and then worsen the severity of their systemic lupus.

In case, the lupus treatment choices are limited to the immunosuppressive drugs to maintain the stability of lupus patients' condition. The fact that most of the drugs are off-labeled (Mak & Tay, 2014) lead the question why there is no enough data for them to be an indication, or on-labeled. It is a need to make some experimental studies to obtain the real target of the drugs, or developing new drugs for lupus.

This is an interesting finding that the experimental models for lupus are highly challenging. The wide range of its triggering factors leads the multiple lupus pathogenesis pathways. One of the established mice model for lupus is Pristane-induced lupus (PIL) mice (Comte et al., 2015). The method provides more complex manifestations than other induction model and also transgenic model. The induction processes lasted for about 6 months in F1 Balb/c mice. However, it is difficult to provide F1 mice in a high number for research. In other case, there is no sufficient data about the modulation of Pristane on the T cell pathway of lupus on non-F1 female BALB/c mice. Therefore, this study aimed to measure some lupus biomarkers based on T cell pathway in non-F1 Female Balb/c PIL mice.

RESEARCH METHODS

Materials

The female Balb/c mice aged 4 weeks were received from LPPT Gadjah Mada University, Indonesia. These mice were pathogen-free species with the certificate number of 352/LP3HP/29/VII/2015. They were housed, randomized, and handled in the Experimental Animal Laboratory of Faculty of Pharmacy,

Universitas Airlangga by using standard maintenance in The Guide of the Care and Use of Laboratory Animals 8th edition, published by National Research Council.

TMPD (Pristane) with the code number of Sigma-P2870 was obtained from the Sigma-Aldrich supplier in Singapore. Mouse anti-Sm ELISA Kit was obtained from Cusabio with the code number of CSB-E15976m. Anti-CD4, anti-CD25, anti-CD62L, anti-IFN-α antibodies, and phycoetrin (PE) fluorescent dye were obtained from Biogenesis, USA. Turk reagent, PBS, and aquadest were obtained from the laboratory of Faculty of Pharmacy, Universitas Airlangga, Indonesia.

Methods

The female Balb/c mice used were 7 weeks old when treated by means of 0.5 mL TMPD. The administration was an intraperitoneal injection. The experimental groups consist of 5 TMPD-treated mice and 5 normal mice. This experiment lasted for 6 months (Comte et al., 2015). The mice behavior was observed daily during this experiment. After the 3rd month, the blood creatinine of the mice was measured by using photometry method. After 6 months, the mice were sacrificed. The bone marrow of each experimental mouse was prepared for IFN-α measurement by using flow cytometry. The fresh spleen of each mouse was prepared to be spleen cells that were ready for flow cytometry assay. T regulatory cells (T regs) $CD4^+CD62L^+$ and $CD4^+CD25^+$ were measured by using phycoethrin (PE) staining in flow cytometer BD FACS Calibur. Then, the data was analyzed by using BD CellQuest program. Total leukocyte count (TLC) was manually counted from the plasma by using hemocytometer improved Neubauer. Anti-Sm antibody in plasma was measured by using indirect Enzyme-Linked Immunosorbent Assay (ELISA). The renal histology was observed by using hematoxylin-eosin (HE) staining. The pictures were captured by using microscope Nikon Eclipse Ci, camera DS F12, and NISBR410 program at the magnitude of 400x for kidney observation; and microscope Optilab for joint observation. All of the data was analyzed by using Independent T-test by means of SPSS Statistics (version 22). The ethical clearance of this research was approved by local ICUC of Faculty of Veterinary Medicine, Universitas Airlangga with the certificate number of 526-KE.

RESULTS AND DISCUSSION

RESULTS

The T-cell-dependent pathway manifestations of lupus which are expected from the animal model are the imbalance of the regulatory function, such as the T regulatory marked by CD25 and CD62L/L-selectin. The imbalance leads the increase expression of some cytokines (Liu et al., 2013). Reeves et al., (2009) states that the IFN-α is the main cytokine expressed after the TMPD induction which causes a dangerous signal environment in immune homeostasis. Thus, the mechanism of systemic inflammation process occurs causes the increase of the total leukocytes. The lupus-specific antibodies such as anti-Sm occur in a high probability of 80% of all induced mice. This probability is higher than other lupus-specific antibodies occurred in TMPD induced mice. At last, the antibodies bind antigens to form immune complexes which are attached by a glomerular basement membrane (GBM) of the kidney (Nielsen et al., 2016). Therefore, in this research, we observed the T regulatory $CD4^+CD25^+FOXP3^+$, $CD4^+CD62L^+$, IFN-α, total leukocyte, anti-Sm antibody, and the structural damage of the renal tissue.

T regulatory cells (T regs)

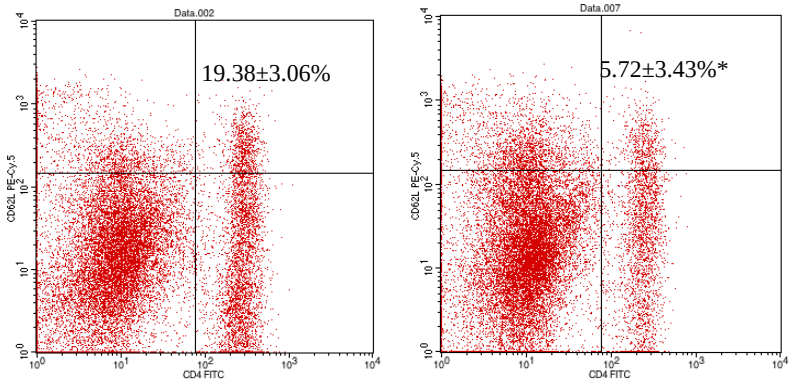
T-regs have several markers on the extracellular and intracellular site. These markers include the positive expressions of CD25, CTLA-4, GITR, OX40, and L-selectin (CD62L) (Rifa'i, 2011; Yagi et al., 2004) T regs measured in this experiment were $CD4^+CD25^+$, $CD4^+CD62L^+$, and $CD25^+FOXP3^+$ T cells from fresh spleen cells. The results are shown in Figure 1, 2, and Table 1.

Table 1. The percentage profile of $CD25^+FOXP3^+$ T regs

Group	The percentage profile of $CD25^+FOXP3^+$ (%)±SD
Naïve	10.32±4.46
TMPD-treated	7.70±4.46*

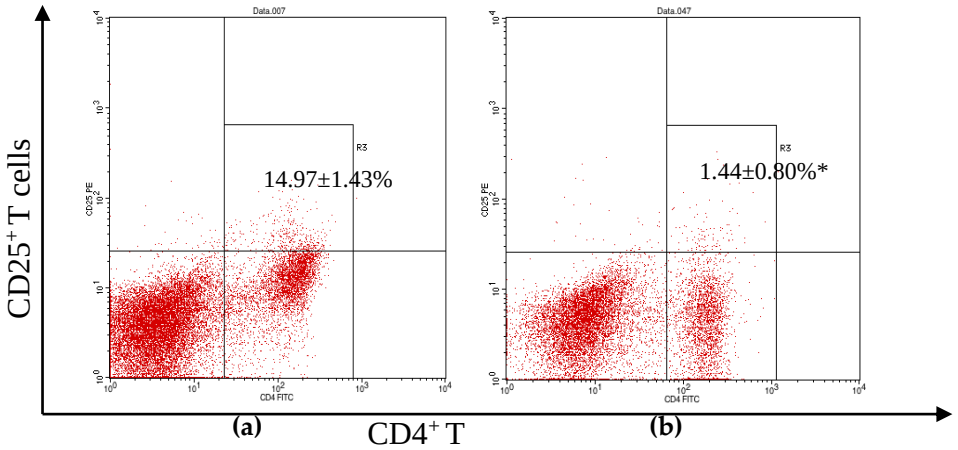
The results show that TMPD reduces the number of $CD4^+CD62L^+$ and $CD4^+CD25^+$ T regs significantly ($p<0.05$). The results were supported by the FOXP3 result as a specific marker of T cells. The low number of T-regs reveals the lupus manifestation as mentioned by (Kluger et al., 2016), FOXP3 T regs found in a low number in lupus mice. The data reveal the T regs condition in human lupus which has the low number of T regs related cytokines. Normally, T regs represent 5-10% of $CD4^+$

in both human and mice (Suen & Chiang, 2012). The low number of T regs will lead to the unregulated T cell effector. It causes the auto-reactive T cells, which cannot be handled by its regulator, attack the normal cells.



(a) (b)

Figure 1. The profile of $CD4^+CD62L^+$ T regs in the spleen cells of the naïve mouse (a) and TMPD-treated mouse (b) analyzed by using flow cytometry

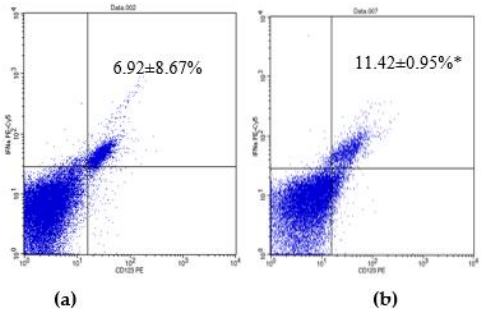


(a) (b)

Figure 2. The profile of $CD4^+CD25^+$ T regs on spleen cells of naïve mouse (a) and TMPD treated mouse (b) analyzed by using flow cytometry

IFN-α

Interferon (IFN) type I especially IFN-α has an important role in the direct and indirect processes of autoantibody formation (Rottman & Willis, 2010). The total IFN-α measured in the normal and TMPD-treated mice is shown in Figure 4. The relative percentages of IFN-α in TMPD-treated mice increase significantly ($p<0.05$) indicates the increase of the feedback-loop mechanism results in the excessive production of auto-antibodies by B cells. The flow cytometry assay was performed by means of dendritic cells (DCs) from the marrow-bone of the femur bone of the tested mice as the sample. DCs and its subset, plasmacytoid dendritic cells (pDCs), can release large amounts of IFN-α and β to control immune responses. The increase of IFN-α causes the activation of auto-reactive T cells, and then it induces the B cells to produce lupus-specific antibodies which lead the severity of lupus manifestations (Stone et al., 2012).



(a) (b)

Figure 3. The profile of $CD123^{+}IFN\text{-}\alpha^{+}$ Tregs in dendritic cells of the naïve mouse (a) and TMPD-treated mouse (b) analyzed by using flow cytometry

Blood disorder

In this experiment, the blood parameter was focused on the total leukocyte as the additional parameter in this T-cell-dependent pathway. The total leukocyte count is a general parameter that indicates an inflammation or even an infection. The result of the total leukocyte count (TLC) of each group on the 6th month after the induction is on Table 2. This result shows that the total leukocytes increase significantly ($p<0.05$) in the TMPD-treated mice. It is a reflection of a systemic inflammation usually occurs in systemic lupus.

Table 2. The total leukocyte count (TLC) of normal and TMPD-treated mice

Group	The Total Leukocyte Count (cells/mm³)
Naïve	9,800 ± 1,698
TMPD-treated	17,500 ± 1,490*

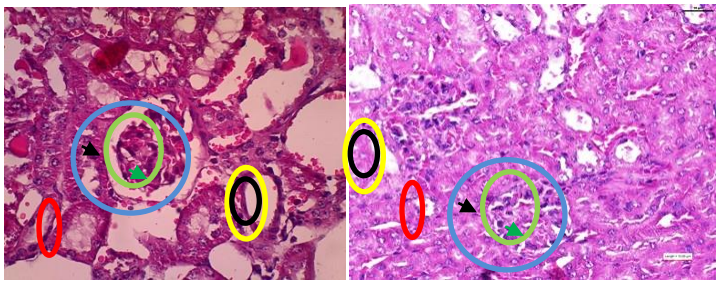
* Significantly different to the naïve group ($p<0.05$)

Lupus-specific antibodies formation

The lupus-specific antibodies formation process occurs slowly. On the 2nd month after the induction, anti-nuclear antibody (ANA) occurs. On the 3rd month, anti-Smith/RNP antibody occurs (Reeves et al., 2009) (Reeves et al., 2009). Then, on the 6th-month anti-dsDNA antibody occurs In this experiment, the anti-Sm antibody occurred on the 6thmonth of the induction. It had a high level with a mean of 109.93±12.02 pg/mL measured by means of Anti-Sm ELISA kit for mice. The high level of lupus-specific antibodies combined with the role of toll-like receptor (TLR) 9 and TLR 4 cause organ injury in TMPD-treated mice as a lupus manifestation (Summers et al., 2010).

Renal disorder parameters

The renal disorder that led to a nephritis manifestation was measured by using renal histology observation. After the 6th month of induction, the mice were sacrificed. The histology of renal tissues was observed. There were many changes in tubules and glomerulus area. The results are shown in Figure 4. The TMPD-treated mice shows an inflamed glomerulus (blue circle), abnormal tubules (yellow circle), a colored proteinaceous cast (black circle), the lysis of epithelial cells of tubules (red circle), accumulation of inflammatory cells in the glomerulus (green circle), the thickening of glomerular basement membrane (GBM) (black arrow), and the thickening of mesangium area (green arrow). Glomerula are located in the cortex area. In the medulla, there are ductus and tubules. The Figure 4b shows the appearance of mice kidney tissue which is damage caused by an autoimmune mechanism. The assessment was performed based on the classification published before (Moroni et al., 2016).



(a) (b)

Figure 4. Observation of renal tissue from naïve mouse (a) and TMPD-treated mouse (b) with 4-6 μm thickness and the magnitude of 400x by using microscope Nikon Eclipse Ci

Compared to the naïve (Figure 4a), the glomerulus of the TMPD-treated mice is inflamed so the Bowman's capsule spaces looked narrower than the naïve mice. The inflammatory is marked by the proliferation of cells in the glomerulus, especially inflammatory cells. The glomerular basement membrane (GBM) area looks thick until it attached to Bowman's capsule. The glomerulus is a complex of capillaries so the GBM is not limited at the outer side of the glomerulus. When it attached, the filtration function will be reduced.

Beside the glomerulus, there are a low number of the epithelial cells in the tubules. The staining cannot show the specific tubules proximal or distal since the proximal has silia inside the ductus. In the tubules, most of the epithelial cells are

at a stage of pyknosis, karyorrhexis, and lysis. In the tubules lumen, there is proteinaceous casts that are colored by HE staining become slightly red depends on the protein remains on the cast. In the naïve mice, the protein was absorbed into the circulation and not remains in the tubules.

At last, the TMPD-treated mice show proliferations of mesangial cells (green arrow) as a sign of severe inflammation. The changes in renal tissue show lupus nephritis signs according to Moroni et al., (2016) which reports the structural changes in the renal biopsy of lupus patient from the mild until the severe tissue damages.

DISCUSSION

Animal models of lupus that are obtained by using induction method are quite rare. A model that has similar manifestation as human lupus is induction method by using TMPD. 0,5 mL of TMPD is injected intraperitoneally into mice under aseptic condition. The severe imbalance of the immune system including biomarkers in T-cell dependent pathway of lupus is obtained on 6 months In this experiment, the manifestation occurred very slowly so it was considered to give a booster injection on the 90th day. The TMPD used was a free impurities material with >98% purity. The wrong choice of the TMPD product would result in an anaphylactic shock in mice about 3 minutes after injection.

Biological variations of mice would affect the induction time. One of them is a variation of expression of neutrophil gelatinase-associated lipocalin (NGAL) NGAL . In this research, the mice were selected strictly so the biological variations could be minimized. On daily observation, a few ascites mice found in the 2nd-month of induction. This ascites condition would recover for several days under a standard daily maintenance. The mice that could not survive develop an excessive accumulation of gasses in the peritoneal cavity. The ascites was formed under the effect of TMPD mechanism, not a manifestation of an infection. The TMPD also impacted on increasing of renal and spleen organ indexes. This increase did not occur in the liver. The result of the other biomarkers was described below.

1. T regs

T-regs have a regulation function that affects on immune homeostatic (Scheinecker et al., 2010). The $CD4^+CD25^+$ T cells area good markers of T regs but not specific because it is also produced by other cells, so that it needs FOXP3 as supporting marker. The $CD25^+FOXP3^+$ is a specific marker of T cells. T regs used in this experiment was CD4 by using $CD25^+$ (α receptor of IL-2) and CD62L (L-selectin) as the surface markers. The results are shown in Table 1-3 and Figure 1-2. There was a significant reduction of the percentages of $CD4^+CD25^+$ and $CD4^+CD62L^+$ T regs. These data supported by the reduction of the $CD25^+FOXP3^+$ percentages in TMPD-treated mice. These data shows an autoimmune impact.

2. IFN-α

In this research, the samples were obtained from the marrow-bone indicated that IFN-α produced by plasmacytoid dendritic cells (pDC) with CD123 as the surface marker. The result (Figure 3) shows a significantly increased level of IFN-α in the TMPD-treated mice. IFN-α is a good biomarker for drug testing. IFN-α is a type I interferon which the position is at the cross-roads between innate and adaptive immunity that leads to initiate and perpetuate lupus (Meyer, 2012; Yang et al., 2012). In the adaptive immunity, IFN-α effects on blunting of the Treg FOXP3 response. IFN-α overproduction in lupus is related to both genetic and environmental factors. It is due mainly to immature plasmacytoid dendritic cells (pDCs) that selectively express TLR-7 and TLR-9 (Richez et al., 2011).

3. Total leukocyte

The total leukocyte is a marker of an abnormal condition such as infection and inflammation. It is not specific for lupus but is sufficient as inflammation marker on systemic lupus. The total leukocyte count significantly increased in the TMPD-treated mice on the 6th month (Table 3). It indicates that the imbalance of immune system leads to tissue and organ damage. This organ damage is an impact of immune complex deposits. Although leukocyte was not specific, it could be a marker that easily assessed during induction time without sacrificing the mice. This data would be better than proteinuria semi-quantitative data.

4. Anti-Sm antibody

The TMPD is a hydrocarbon that could induce specific autoantibodies, such as anti-nRNP, Sm, Su, and dsDNA (Summers et al., 2010). It meets the lupus criteria as occurs in human (Yu et al., 2014). In this experiment, the ELISA result shows that anti-Sm antibody was present in TMPD-treated mice. It was supported by the previously result (Adnyana et al., 2014) states that ANA present on the 2nd month of induction. These antibodies would make immune complex that could harm the tissues and organs.

5. Renal disorder

It needs about 6 months to obtain the immune complex formation in the kidney that leads to nephritis in mice. The previous studyresults in the increased level of protein in the urine occurs after 6

months of TMPD induction in mice. The renal disorder caused by TMPD was clearly explained in the results. The same structural kidney disorder is also found in the previous research (Aparicio-Soto et al., 2016; Lin et al., 2017).

Study Limitation

There are some limitations of the TMPD-treated mice as a lupus animal model. A wide range of data was obtained as an impact of biological variations. We could not make the same baseline of manifestation severity on each induced mouse used so that the research needed a lot of replication. For further research, it is a necessary to find a method that could measure the baseline of ready-use TMPD-treated mice for drug testing. The method could minimize the deviation standard. The NGAL parameter could be one of the considered biomarkers.

The mice strains for this TMPD-induction method used were limited to the female Balb/c mice or some strains that immune-manipulated. The other publications report that a long-term induction time (about 6 months) is not suitable for mice that only live for 1.5-2 years (Urbonaviciute et al., 2013; Zhu et al., 2015, 2017).

Chance for the future development

Another TMPD manifestation in this research that was not explained in the previous reports was the effect on the neural function. After the 6th month, we found a severe neural imbalance manifestation caused the mice walking in a circle for several days. This evident was quite rare and the signs only lasted no more than 7 days. It was a challenge for the next development to find a mechanism of TMPD on the neural manifestation of lupus.

CONCLUSION

Pristane or TMPD modulates specific changes on $CD4^+CD25^+$ and $CD4^+CD62L^+$ T regs, IFN-α, and then impacts on anti-Sm antibody specific lupus, increase of total leucocyte in non BALB/c F1 mice. The pathway makes a renal structural damages as lupus nephritis signs occurred in human. Further research to determine other markers are necessary.

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BIBLIOGRAPHY

Adnyana, I. K., Yulinah, E., Maeistuti, N., & Setiawan, F. (2014). Evaluation of Ethanolic Extracts of Mullaca (Physalis Angulata L.) Herbs for Treatment of Lupus Disease in Mice Induced Pristane. *Procedia Chem*, *13*, 186–193.

Aparicio-Soto, M., Sánchez-Hidalgo, M., Cárdeno, A., Rosillo, M. Á., Sánchez-Fidalgo, S., Utrilla, J., Martín-Lacave, I., & Alarcón-de-la-Lastra, C. (2016). Dietary extra virgin olive oil attenuates kidney injury in pristane-induced SLE model via activation of HO-1/Nrf-2 antioxidant pathway and suppression of JAK/STAT, NF-kB and MAPK activation. *J Nutr Biochem*, *27*, 278–288.

Comte, D. K., P, M., & GC, T. (2015). T cells as a therapeutic target in SLE. *Lupus*, *24*, 351–363.

Kluger, M., Nosko, A., Goerke, B., Luig, M., Stahl, R. A. K., & Steinmetz, O. M. (2016). P24 RORÎ3t+ FOXP3+ BITREGS PROMOTE LUPUS NEPHRITIS VIA IL-17 SECRETION AND SUPPRESSION OF TH2 RESPONSES. *Kidney International Reports*, *1*(4), S11–S12.

Lin, Y., Yan, Y., Zhang, H., Chen, Y., He, Y., Wang, S., Fang, L., Lv, Y., & Du, G. (2017). Salvianolic acid A alleviates renal injury in systemic lupus erythematosus induced by pristane in BALB/c mice. *Acta Pharmaceutica Sinica B*, *7*(2), 159–166.

Liu, X., Fang, L., Guo, T. B., Mei, H., & Zhang, J. Z. (2013). Drug targets in the cytokine universe for autoimmune disease. *Trends in Immunology*, *34*(3), 120–128.

Mak, A., & Tay, S. H. (2014). Environmental factors, toxicants and systemic lupus erythematosus. *International Journal of Molecular Sciences*, *15*(9), 16043–16056.

Meyer, O. (2012). Interferon-alpha as a treatment target in systemic lupus erythematosus. In *Joint bone spine* (Vol. 79, Issue 2, pp. 113–116).

Moroni, G., Depetri, F., & Ponticelli, C. (2016). Lupus nephritis: when and how often to biopsy and what does it mean? *Journal of Autoimmunity*, *74*, 27–40.

Nielsen, C. T., Rasmussen, N. S., Heegaard, N. H. H., & Jacobsen, S. (2016). "Kill" the messenger: targeting of cell-derived microparticles in lupus nephritis. *Autoimmunity Reviews*, *15*(7), 719–725.

Reeves, W. H., Lee, P. Y., Weinstein, J. S., Satoh, M., & Lu, L. (2009). Induction of autoimmunity by

pristane and other naturally occurring hydrocarbons. *Trends in Immunology*, *30*(9), 455–464.

Richez, C., Blanco, P., Rifkin, I., Moreau, J.-F., & Schaeverbeke, T. (2011). Role for toll-like receptors in autoimmune disease: the example of systemic lupus erythematosus. *Joint Bone Spine*, *78*(2), 124–130.

Rifa'i, M. (2011). Protective Effect of CD4+ CD25+ Regulatory T Cells on Mice Model of Rheumatoid Arthritis. *Annales Bogorienses*, *15*(1), 1–6.

Rottman, J. B., & Willis, C. R. (2010). Mouse models of systemic lupus erythematosus reveal a complex pathogenesis. *Veterinary Pathology*, *47*(4), 664–676.

Scheinecker, C., Bonelli, M., & Smolen, J. S. (2010). Pathogenetic aspects of systemic lupus erythematosus with an emphasis on regulatory T cells. *Journal of Autoimmunity*, *35*(3), 269–275.

Stone, R. C., Feng, D., Deng, J., Singh, S., Yang, L., Fitzgerald-Bocarsly, P., Eloranta, M., Rönnblom, L., & Barnes, B. J. (2012). Interferon regulatory factor 5 activation in monocytes of systemic lupus erythematosus patients is triggered by circulating autoantigens independent of type I interferons. *Arthritis & Rheumatism*, *64*(3), 788–798.

Suen, J.-L., & Chiang, B.-L. (2012). CD4+ FoxP3+ regulatory T-cells in human systemic lupus erythematosus. *Journal of the Formosan Medical Association*, *111*(9), 465–470.

Summers, S. A., Hoi, A., Steinmetz, O. M., O'Sullivan, K. M., Ooi, J. D., Odobasic, D., Akira, S., Kitching, A. R., & Holdsworth, S. R. (2010). TLR9 and TLR4 are required for the development of autoimmunity and lupus nephritis in pristane nephropathy. *Journal of Autoimmunity*, *35*(4), 291–298.

Urbonaviciute, V., Starke, C., Pirschel, W., Pohle, S., Frey, S., Daniel, C., Amann, K., Schett, G., Herrmann, M., & Voll, R. E. (2013). Toll-like receptor 2 is required for autoantibody production and development of renal disease in pristane-induced lupus. *Arthritis & Rheumatism*, *65*(6), 1612–1623.

Yagi, H., Nomura, T., Nakamura, K., Yamazaki, S., Kitawaki, T., Hori, S., Maeda, M., Onodera, M., Uchiyama, T., & Fujii, S. (2004). Crucial role of FOXP3 in the development and function of human CD25+ CD4+ regulatory T cells. *International Immunology*, *16*(11), 1643–1656.

Yang, L., Feng, D., Bi, X., Stone, R. C., & Barnes, B. J. (2012). Monocytes from Irf5−/− mice have an intrinsic defect in their response to pristane-induced lupus. *The Journal of Immunology*, *189*(7), 3741–3750.

Yu, C., Gershwin, M. E., & Chang, C. (2014). Diagnostic criteria for systemic lupus erythematosus: a critical review. *Journal of Autoimmunity*, *48*, 10–13.

Zhu, W., Jiang, C., Xu, J., Geng, M., Wu, X., Sun, J., Ma, J., Holmdahl, R., Meng, L., & Lu, S. (2015). Pristane primed rat T cells enhance TLR3 expression of fibroblast-like synoviocytes via TNF-α initiated p38 MAPK and NF-κB pathways. *Clinical Immunology*, *156*(2), 141–153.

Zhu, W., Xu, J., Jiang, C., Wang, B., Geng, M., Wu, X., Hussain, N., Gao, N., Han, Y., & Li, D. (2017). Pristane induces autophagy in macrophages, promoting a STAT1-IRF1-TLR3 pathway and arthritis. *Clinical Immunology*, *175*, 56–68.