รายงานการวิจัย

การศึกษาทางชีววิทยาเชิงระบบของการควบคุมอิมมูนเซลล์ระดับโมเลกุลในหนูโรคลูปัส

Systems biology investigation of immune cell regulation at the molecular level in SLE

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บทคัดย่อภาษาไทย

โรคแพ้ภูมิตนเองเกิดขึ้นเมื่อเซลล์ภูมิคุ้มกันตอบสนองต่อแอนติเจนของตัวเองผิดปกติและนำไปสู่การอักเสบในเนื้อเยื่อ ปฏิสัมพันธ์ระหว่างพันธุกรรมและสิ่งแวดล้อมเป็นส่วนส่งเสริมให้อาการของโรคเป็นมากขึ้น สารไซโตไคน์ที่ทำให้เกิดโรคเอสแอลอีและโรครูมาตอยด์ที่สำคัญคือ อินเตอร์เฟอร์รอนแอลฟ่า โรคเอสแอลอีเกิดจากภูมิต้านทานผิดปกติเรื้อรังซึ่งจะทำให้เกิดการสร้างแอนติบอดี้ต่อต้านเนื้อเยื่อตนเองและทำให้เกิดไตอักเสบ การส่งสัณญาณผ่านเซ็นเซอร์กรดนิวคลีสามารถกระตุ้นการผลิตอินเตอร์เฟอร์รอนแอลฟ่าจากเดนไดรติกเซลล์ และส่งเสริมความรุนแรงของโรคเอสแอลอี Stimulator of interferon genes (Sting) เป็นตัวรับดีเอ็นเอและส่งสัญญาณต่อเพื่อให้เกิดการสร้างอินเตอร์เฟอร์รอนแอลฟ่า ความผิดปกติของ Stingสามารถก่อให้เกิดโรคหลอดเลือดอักเสบในเด็กซึ่งมีอาการคล้ายโรคเอสแอลอี อย่างไรก็ตามการศึกษาการทำงานของ Sting ในสัตว์ทดลองของโรคแพ้ภูมิตนเองยังมีความขัดแย้งกันอยู่ เพื่อที่จะค้นหาว่า Sting มีส่วนร่วมในการเกิดโรคลูปัส, หนูลูปัส Fcgr2b-deficient จะถูกทำการผสมพันธุ์กับหนู Sting-deficient และหนูที่ได้จากการผสมนี้จะถูกติดตามอาการของโรคลูปัส จากผลการศึกษานี้พบว่า หนูลูปัส Fcgr2b-deficient สามารถมีชีวิตอยู่ได้นานขึ้น มีการสร้างแอนติบอดี้ต่อเนื้อเยื่อตนเองลดลง และ เกิดไตอักเสบลดลง ความรู้จากการศึกษานี้เป็นหลักฐานยืนยันแนวคิดสำหรับการวางแผนที่จะยับยั้ง Sting ในการรักษาโรคแพ้ภูมิตนเองในอนาคต

Abstract

Autoimmune diseases occur when the immune cells react against self-antigens and subsequently lead to inflammation in the tissues. The interactions between genetics and environmental triggers regulate the phenotypes and outcome of the diseases. Type I interferon has been shown as one of the most crucial cytokines involving in the pathogenesis of autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). SLE is a chronic systemic autoimmune disease which can result in autoantibody production and fatal glomerulonephritis. Activation via nucleic acid sensors can induce the production of type I interferon from dendritic cells and promote SLE severity. Stimulator of interferon genes (Sting) is a cytoplasmic DNA sensor that signals downstream to enhance type I interferon production after its activation. Recently, it was shown that a gain mutation in the STING gene resulting in over-activity of the IFN pathway can cause familial inflammatory syndrome with lupus-like manifestations in humans. However, the functional studies of Sting in different autoimmune mouse models suggest the conflicting roles of Sting in the pathogenesis of autoimmune diseases. In order to determine if Sting participates in lupus pathogenesis, the Fcgr2b-deficienct mice (lupus mouse model) were bred with Sting-deficient mice to create the double-deficient mice. In the absence of Sting, the Fcgr2b-deficient mice do not develop fatal glomerulonephritis and autoantibodies. The original knowledge from this study is a proof of concept for targeting Sting as a future promising treatment in autoimmune diseases.

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6. คำอธิบายสัญญาลักษณ์และคำย่อที่ใช้ในการวิจัย (List of Abbreviations)

SLE, Systemic lupus Erythematosus; Fcgr2b, Fc gamma receptor IIb; Sting; type I interferon,

ANA	Antinuclear antibody
BCA	Bicinchoninic acid assay
bp	Base pair
BSA	Bovine serum albumin
cDNA	Complementary DNA
CFSE	Carboxyfluorescein succinimidyl ester
cGAMP	Cyclic guanosine monophosphate–adenosine monophosphate
cGAS	Cyclic GMP-AMP synthase
ddCt	Delta delta cycle threshold
DTT	Dithiothreitol
ELISA	Enzyme-linked immunosorbent assay
FA	Formic acid
Fcgr2b	Fc fragment of IgG receptor IIb
IA	Iodoacetamide
Ifi	interferon-inducible Ifi200 family
IFNR	Interferon receptor
Ifn-γ	Interferon-gamma
Ifn-α	Interferon-alpha
Ifn-β	Interferon-beta
Irf3	Interferon regulatory factor 3
Irf5	Interferon regulatory factor 5
Irf7	Interferon regulatory factor 7
IL	Interleukin
IP	Immunoprecipitation
ISG	Interferon-stimulated gene

LC	Liquid chromatography
M	Molar
MHC	Major histocompatibility complex
MS/MS	Tandem mass spectrometry
pDC	Plasmacytoid dendritic cell
qPCR	Quantitative polymerase chain reaction
RT-PCR	Reverse-transcription polymerase chain reaction
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEM	Standard error of the mean
SLE	Systemic lupus erythematosus
Sting	Stimulator of interferon gene
TEAB	Triethylammonium bicarbonate
TEMED	N,N,N',N'-tetramethylethylenediamine
TFA	Trifluoroacetic acid
Tlrs	Toll-like-receptor
Tmem173	Transmembrane 173
WB	Western blotting assay

โครงการวิจัยย่อยที่ 1

“รายงานการวิจัยเรื่อง การศึกษาการควบคุมของวิถีสัญญาณอินเตอร์เฟียรอนผ่านเซ็นเซอร์ของไวรัสดีเอ็นเอในเซลล์ภูมิคุ้มกันของหนูโรคลูปัสด้วยวิธีโปรตีโอมิกส์”

“Systems-investigation of aberrant signaling in immune cells of SLE mouse model”

ส่วนประกอบเนื้อเรื่อง

1. บทนำ (Introduction) ซึ่งกล่าวถึงเนื้อหาของเรื่องที่เคยมีผู้ทำการวิจัยมาก่อน ความสำคัญและที่มาของปัญหา วัตถุประสงค์และขอบเขตการวิจัย วิธีดำเนินการวิจัยโดยสรุปทฤษฎีและ/หรือแนวทางความคิดที่นำมาใช้ในการวิจัย ประโยชน์ที่คาดว่าจะได้รับ ฯลฯ

Fcgr2b เป็น Fc receptor ที่ทำหน้าที่ควบคุมและยับยั้งการทำงานของเซลล์ในร่างกาย เพื่อป้องกันไม่ให้เซลล์ที่ถูกกระตุ้นนั้นตอบสนองต่อตัวกระตุ้นและทำงานเพิ่มขึ้นเพียงอย่างเดียวโดยที่ไม่สามารถกลับเข้าสู่ภาวะปกติได้เมื่อหมดตัวกระตุ้นแล้ว ดังนั้นในหนูที่ปราศจาก Fcgr2b (Fcgr2b-/-) จึงมีการทำงานของเซลล์ที่มากผิดปกติโดยเฉพาะ B เซลล์และ macrophage เซลล์ ซึ่งเป็นผลทำให้เกิดโรค autoimmune ขึ้นได้ (1) ในหนูลูปัส Fcgr2b-/- จะมีอาการแสดงของโรคใกล้เคียงกับผู้ป่วย SLE อย่างมาก (1) และในหนู Fcgr2b-/- ที่มีอายุน้อยก็สามารถทำให้เกิดอาการเหมือนกับโรค Rheumatoid arthritis โดยการฉีด collagen (collagen-induced arthritis) นอกจากนี้ความผิดปกติของยีน Fcgr2b ก็สามารถพบได้ในผู้ป่วยชาวเอเชียรวมทั้งคนไทยที่เป็น SLE (2-4)* ดังนั้นโมเดลนี้จึงเป็นโมเดลที่เหมาะสมสำหรับใช้ในการศึกษากลไกการเกิดโรค autoimmune disease ทั้งโรค SLE และ Rheumatoid arthritis โดยที่ผู้วิจัยเองได้มีประสบการณ์ในการทำงานวิจัยเพื่อที่จะศึกษากลไกการเกิดโรคในหนู Fcgr2b-/- มาแล้ว โดยพบว่า interleukin-17 signaling pathway มีความสำคัญต่อทั้งการเกิด lupus nephritis ในหนูลูปัส Fcgr2b-/- (5) และต่อการเกิด rheumatoid arthritis ในโมเดล collagen-induced arthritis ในหนู Fcgr2b-/- เช่นกัน (6) นอกจากนี้เรายังสามารถใช้หนูลูปัส Fcgr2b-/- ในการวิจัยเพื่อศึกษากลไกการเกิดโรค SLE และนำความรู้ที่ได้นั้นมาประยุกต์ใช้ในการรักษาผู้ป่วยโรค SLE ต่อไป

การแสดงออกของ TLR9 และ TLR7 มีผลต่อการทำให้เกิดโรคลูปัสและการสร้าง autoantibody (7-9) นอกจาก TLRs แล้ว, การเพิ่มมากขึ้นของ RNA-sensing โมเลกุลอย่างเช่น MDA5 ก็สามารถกระตุ้นให้หนูที่เป็นโรคลูปัสอยู่แล้วมีอาการที่รุนแรงขึ้นได้ (10) และความผิดปกติของ DNA metabolism ในหนูที่ขาดยีน Trex-1 (11) และ DNase (12, 13) ก็ทำให้เกิดโรคลูปัสได้เหมือนกัน นอกจากนี้ในผู้ป่วย SLE ก็ตรวจพบว่ามีการสร้างแอนติบอดีต่อ DNA-sensing โมเลกุล IFI16, เชื้อไวรัส HHV8 และ CMV (14-16) อย่างไรก็ตามเป็นที่ทราบกันว่าอาการของโรค SLE จะเพิ่มความรุนแรงขึ้นอย่างมีนัยสำคัญในระหว่างการติดเชื้อจากไวรัสต่างๆ เช่น CMV และ EBV (14, 17) จะเห็นได้ว่าข้อมูลเหล่านี้บ่งชี้ให้เห็นถึงความเชื่อมโยงระหว่างการติดเชื้อไวรัส,

เซ็นเซอร์ของกรดนิวคลีอิกในเซลล์และการเกิดโรคลูปัส แต่ถึงกระนั้นปัจจุบันก็ยังไม่มีการศึกษาที่ทำให้ทราบถึงบทบาทและกลไกของ DNA-sensing โมเลกุลที่อยู่ใน cytoplasm ของเซลล์ว่ามีผลต่อการเกิดโรคลูปัสหรือไม่ และจนถึงปัจจุบันความเข้าใจในกลไกที่ทำให้เกิดปรากฏการณ์นี้ก็ยังไม่ทราบชัดเจน ซึ่งถ้าเราสามารถทำงานวิจัยที่นำมาซึ่งความเข้าใจในกลไกการเกิดโรคนี้ได้ ข้อมูลนี้อาจนำไปสู่ความรู้ขั้นสูงในการพัฒนาวัคซีนรักษาโรคในการรักษาผู้ป่วยโรคลูปัสในอนาคต โดยทั่วไป DNA-sensing โมเลกุลที่ถูกกระตุ้นโดย Interferon (IFNs) จะส่งสัญญาณผ่าน ทางโปรตีน adaptor ที่เรียกว่า STING (Stimulator of interferon genes) (18-20) นอกจากนี้ STING ยังเป็นโมเลกุลที่จำเป็นสำหรับการรวมตัวของไวรัสและ membrane ของ host ซึ่งขบวนการนี้จะกระตุ้นให้เกิดการหลั่ง Interferon (21) ดังนั้น STING จึงเป็นโมเลกุลตัวแทนสำหรับการศึกษาถึงบทบาทของ DNA-sensing pathway ว่ามีผลต่อการเกิดโรคลูปัสหรือไม่

2. เนื้อเรื่อง (Main Body) ซึ่งกล่าวถึงรายละเอียดเกี่ยวกับวิธีดำเนินการวิจัย (Materials & Method)

ผลการวิจัย (Results) ฯลฯ

Materials and Methods

Animal and animal models

Fcgr2b-/- mice on C57BL/6 background were provided by Bolland S. (NIH, Maryland, USA). Sting-/- mice were provided by Paludan (Aarhus University, Aarhus, Denmark). Wild type mice were purchased from the National Laboratory Animal Center, Nakornpathom, Thailand. The Fcgr2b-/- mice were bred with Sting-/- mice to create double deficient mice and their littermate controls. The animal protocols were approved by Faculty of Medicine, Chulalongkorn University followed the National Institutes of Health (NIH) criteria.

Survival study

The double deficient mice will be aged and observed the survival rates compared to their littermates. If the mice can survive up to 12 months, the mice will be euthanized to collect the tissues (kidney, spleen, bone marrow, and sera) for further analysis.

Flow cytometry analysis

The collected spleens were harvested and incubated with collagenase D at 37 c for 30 minutes to isolate splenocyte. The splenocytes were stained with flow antibody. The flow cytometry analysis was performed using BD LSR-II and FlowJo software. The dead cells were excluded from the analysis.

Autoantibody testing

The collected sera of the mice at the age of 6 months were tested for anti-nuclear antibody (Hep-2 cells; immunofluorescence) and anti-dsDNA (ELISA). The sera were diluted into different dilution factors.

Histopathology

The kidneys were fixed in 4%paraformaldehyde and subsequently were stained with H&E and PAS. The pathology grading will be blinded analysis by the experience nephrologist.

Real-time PCR

The RNA from kidneys was isolated using Trizol. The DNase-treated RNA was purified using RNeasy isolation kit. The conversion of RNA to cDNA using iScript RT Supermix (Biorad) was performed. The gene expression profiles were tested using SsoAdvanced Universal SYBR Green Supermix. The sequences of primers tested are followed:

Primer name	Sequence
CXCL10	F: 5'- ATGACGGGCCAGTGAGAATG-3' R: 5'- TCGTGGCAATGATCTCAACAC-3'
Mx1	F: 5'-GATCCGACTTCACTTCCAGATGG-3' R: 5'-CATCTCAGTGGTAGTCAACCC-3'
ISG15	F: 5'-TCTGACTGTGAGAGCAAGCAG-3' R: 5'-ACCTTTAGGTCCCAGGCCATT-3'
IRF3	F: 5'- GCTTGTGATGGTCAAGGTTGT-3' R: 5'- AGATGTGCAAGTCCACGGTT-3'
IRF5	F: 5'-TTTGAGATCTTCTTTTGCTTTGGA-3' R: 5'-GTACCACCTGTACAGTAATGAGCTTCTT-3'
IRF7	F: 5'-CCCAGACTGCCTGTGTAGACG-3' R: 5'-CCAGTCTCCAAACAGCACTCG-3'
IFN-β	F: 5'- GCTTGTGATGGTCAAGGTTGT-3' R: 5'- AGATGTGCAAGTCCACGGTT-3'
IFN-γ	F: 5'- ACT GAC TTG AAT GTC CAA CGC A-3' R: 5'- ATC TGA CTC CTT TTT CGC TTC C-3'

P202	F: 5'-AGC CTC TCC TGG ACC TAA CA-3' R: 5'- GCA GTG AGT ACC ATC ACT GTC A-3'
Sting	F: 5'-TCTGACTGTGAGAGCAAGCAG-3' R: 5'-ACCTTTAGGTCCCAGGCCATT-3'
β- actin	F: 5'-TAGCACCATGAAGATCAAGAT-3' R: 5'-CCGATCCACACAGAGTACTT-3'

Bone marrow-derived dendritic cell (BMDC)

Dendritic cells were obtained from mice' femur bone (BMDC) that had been euthanized by cervical dislocation. Cells were cultured in RPMI 1640 supplement with 10 % FBS, 1 mM Na pyruvate, 10 mM Hepes buffer, 1X L-glutamine, 1X non-essential amino acid, 100 Units/ml of pen/strep (Gibco-Thermo Fisher Scientific, MA USA) and 50 μM of 2-Mercaptoethanol (Sigma-Aldrich, Darmstadt, Germany) and maintained at 37°C in a CO_2 incubator then stimulated with 20 ng/ml of IL4 and 20 ng/ml of GM-CSF (Miltenyi, Bergisch Gladbach, Germany) for 5 days followed by adding 10 ug/ml of DMXAA (5,6-Dimethylxanthenone-4-acetic acid or STING ligand) (Invivogen, San Diego, USA) for 24 hours. The mature BMDC were stained with the following antibodies: anti- mPDCA, CD80, I-Ab, CD11b and CD11c (Biolegend, San Diego, CA, USA). The stained cells will be subjected to flow cytometer (BD Biosciences) and analyzed by FlowJo software (version 10, USA).

Determination of Cytokine in BMDCs

BMDC cells were cultured in 6-well plate and stimulated with IL4 and GM-CSF for 5 days and followed by 10 ug/ml of DMXAA (STING ligand) for 24 hours. The supernatants were collected, and the concentration of cytokines panel including: IL-1α, IL-1β, IL-6, IL-10, IL-12p70, IL-17A, IL-23, IL-27, MCP-1, IFN-β, IFN-γ, TNF-α, and GM-CSF were measured by LEGENDplex™ Mouse Inflammation Panel kit (Biolegend, San Diego, CA, USA) as describe above.

Sample preparation for MS analysis

Quantitative proteomic analysis of mature BMDC was studied using a dimethyl labeling method (73). Briefly, BMDC were cultured, and then stimulated with 10 ug/ml of DMXAA (STING ligand) for 24 hours. Three hundred microgram proteins per group from BMDC were digested overnight at 37°C with trypsin [1:50 (w/w)]. Next, stimulated BMDC's peptides from WT mice,

Fcgr2b$^{-/-}$ mice, and *Fcgr2b*$^{-/-}$*.Sting*$^{gt/gt}$ mice were labeled with light reagents [formaldehyde (Sigma) and sodium cyanoborohydride (Sigma)], medium reagents [formaldehyde-d2 (CIL) and sodium cyanoborohydride], and heavy reagents [deuterated and ^{13}C-labeled formaldehyde (Sigma) and cyanoborodeuteride (CIL)], respectively, for an hour at room temperature. The peptides were fractionated and subjected to LC-MS/MS (Thermo). Significantly differentially regulated proteins were determined by unpaired t-tests with p-value < 0.05 considered significant. The online resource Database for Annotation, Visualization and Integrated Discovery (DAVID, v 6.8, https://david.ncifcrf.gov/) was employed to classify the significant proteins into functional categories using all proteins identified by MS as background.

Western Blot Analysis

Splenocytes were lysed in 2 % SDS lysis buffer. Lysates were homogenized and centrifuged at 12,000×g for 15 min at 4^{o}C. The supernatants were collected, and total protein was measured by BCA protein assay (Thermo Scientific, Illinois, USA). Cell lysates containing equal amounts of protein (20 μg) were boiled in SDS sample buffer at 37^{o}C for 15 min before separation on a 10 % SDS-polyacrylamide gel. Proteins were transferred to nitrocellulose membranes and Western blot analysis.

Adoptive transfer

BMDCs from WT, *Fcgr2b*$^{-/-}$ and *Fcgr2b*$^{-/-}$*.Stin*$^{gt/gt}$ mice were cultured as described above. The recipient *Fcgr2b*$^{-/-}$*.Stin*$^{gt/gt}$ mice (at the age of 4 months) received approximately 10^6 cells of BMDC by tail vein every two weeks per injection time. Control *Fcgr2b*$^{-/-}$*.Stin*$^{gt/gt}$ mice received only sterile PBS (vehicle). Sera were collected and the levels of anti-dsDNA were measured by ELISA. Mice were euthanized 2 weeks after the final transfer (at the age of 6 months).

Statistical analysis

Significant difference of survival rates is tested by Log-rank test. The comparison between groups is examined by T-test.

Result

1. Increase of survival rates of the Fcgr2b-deficient mice in the absence of Sting

The Fcgr2b-deficient mice start to die at the age of 6 months and the survival rates drop to 22.2% by 12 month old while the survival rates of double deficient mice are 77.7% ($p<0.001$). The effect of one allele of Sting to survival rates of Fcgr2b-deficient mice does not show significant difference ($p=0.6$) (Figure 1).

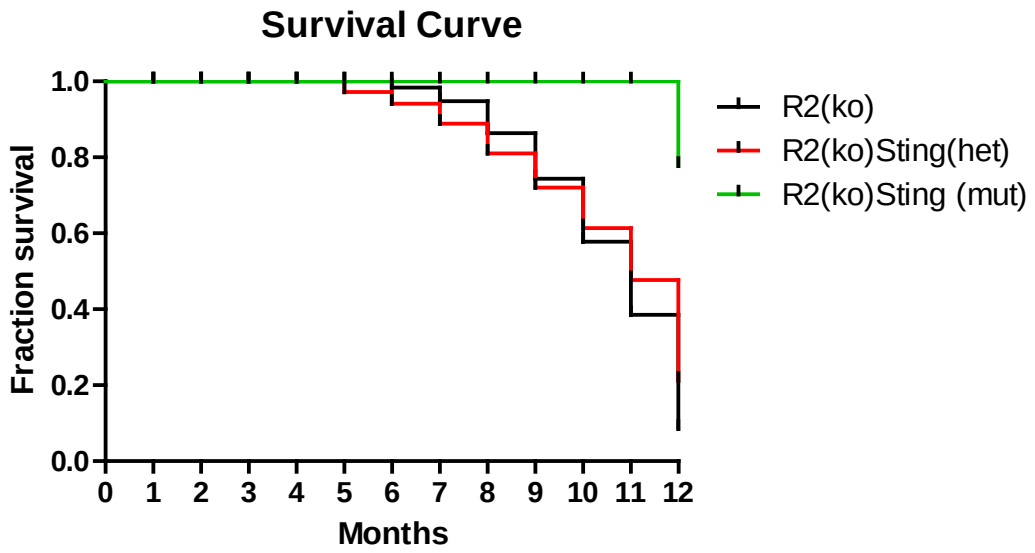

Figure 1 Increase of survival rates of the Fcgr2b-deficient mice in the absence of Sting

The survival of the mice was observed until 12 month old. The absence of Sting increase survival of Fcgr2b-/- mice ($p<0.001$, N=9 per group).

2. Sting deficiency diminishes fatal glomerulonephritis in the Fcgr2b-deficient mice

The kidney staining of Fcgr2b-deficient mice shows inflammatory cell infiltrations, enlarged glomeruli and crescentic glomeruli. In the absence of Sting, the Fcgr2b-deficient mice do not develop glomerulonephritis (Figure 2).

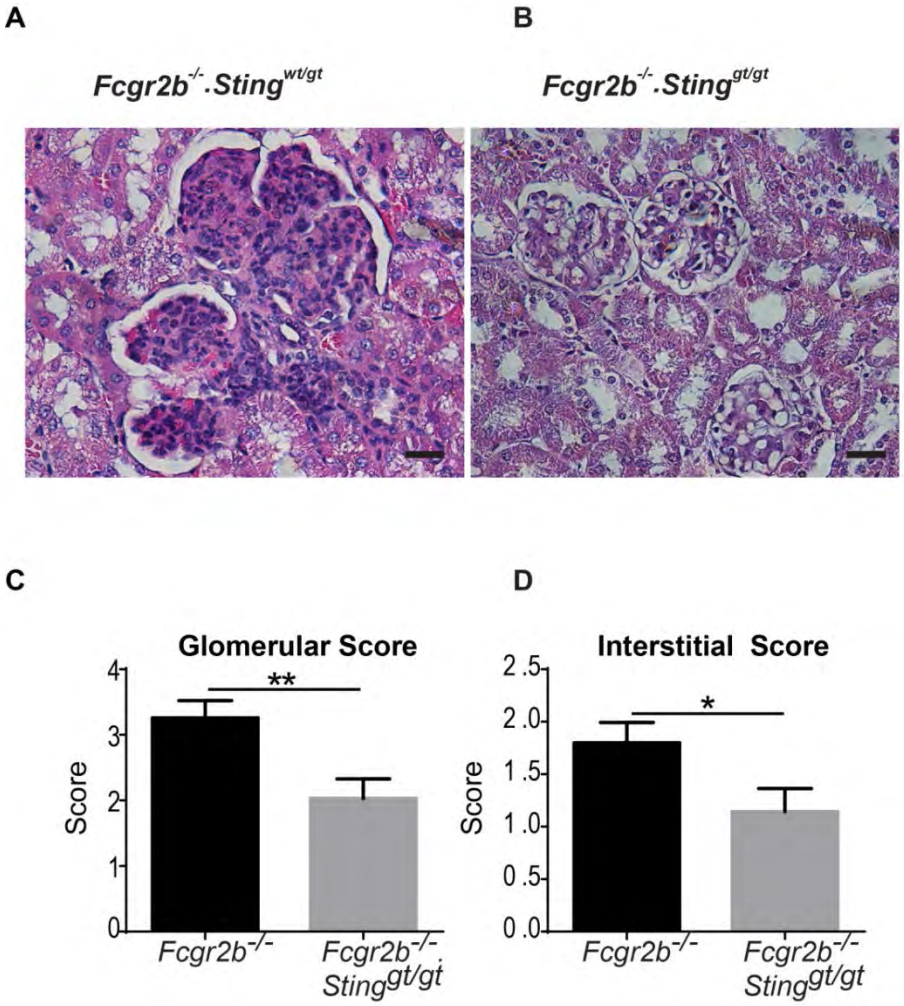

Figure 2 Sting deficiency diminishes fatal glomerulonephritis in the Fcgr2b-deficient mice

(A-B) Kidney sections of *Fcgr2b*$^{-/-}$ and *Fcgr2b*$^{-/-}$. *Sting*$^{gt/gt}$ mice (6-8 months old) were stained with H&E. Data are representative of 7-10 mice per group (scale bar = 25 μm). (C-D) Glomerular scores and interstitial scores of kidney sections were blindly graded (N=7–10 per group). Data show as mean ± SEM (*p < 0.05, **p<0.01 and ***p<0.001

3. Decrease autoantibody production in the double-deficient mice

Anti-dsDNA production and Antinuclear antibody (ANA) decreased in the Sting and Fcgr2b double-deficient mice (Figure 3A-3D).

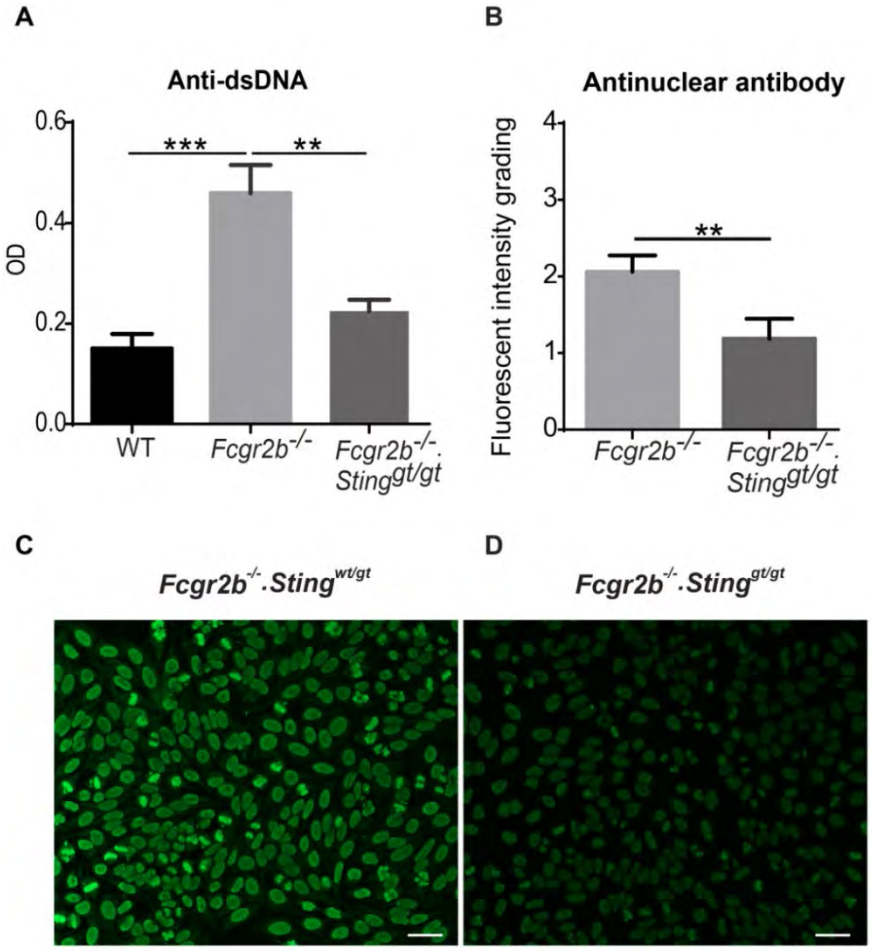

Figure 3 Decrease autoantibody productions in the double-deficient mice

The sera of the mice were collected at the age of 6 months to test for antinuclear antibody (Figure 3A, representative of mice (N=3/group) and anti-dsDNA (Figure 3B, N=6/group, $p<0.01$)

4. Decrease IgG deposition and leukocyte infiltration in the kidneys of the double-deficient mice

The kidney section showed that immune complex deposition (IgG) and leukocyte (CD45) infiltration increased in the Fcgr2b-deficient mice and reduced in double-deficient mice (Figure 4)

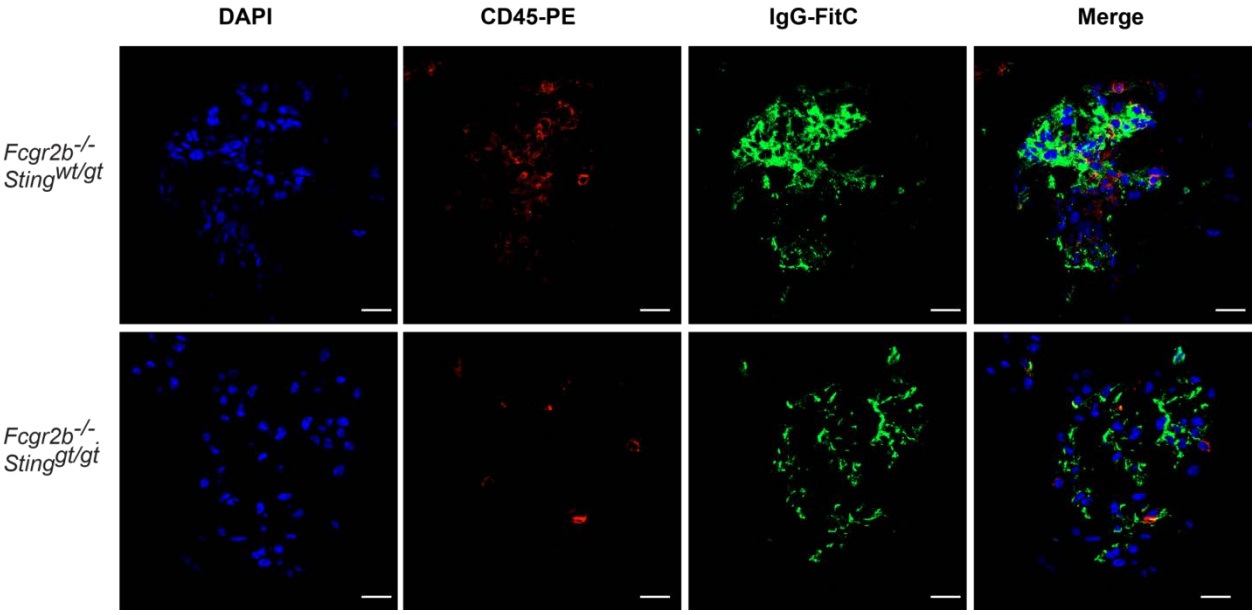

Figure 4 Decrease IgG deposition and leukocyte infiltration in the kidneys of the double-deficient mice.

Immunofluorescence staining in the kidney of *Fcgr2b*$^{-/-}$ and double-deficient mice by confocal microscope (DAPI in blue, CD45 in red and IgG in green). Shown as a representative of mice (N=3 mice per group, scale bar=10 um).

5. Decrease interferon-inducible gene expression in the kidneys of the double-deficient mice

Sting-mediated signaling induces type I interferon production and leads to the increase of interferon inducible gene expression. We determined whether the interferon signature genes in the kidneys will diminish in the Fcgr2b-double deficient mice. The results show that IRF3, IRF5, IRF7 and Mx1 expression were decreased in the double-deficient mice compared to Fcgr2b-deficient mice (Figure 4).

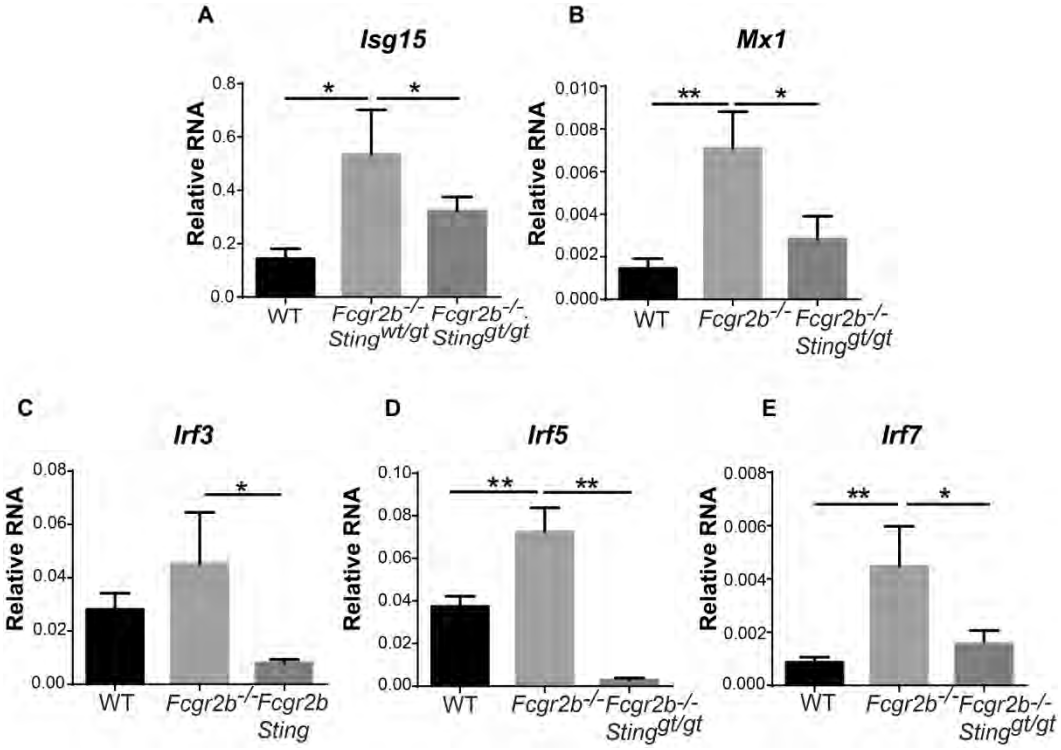

Figure 5 Decrease interferon-inducible gene expressions in the kidneys of the double-deficient mice.

(A-E) Gene expression profiles from the kidneys of wild-type, *Fcgr2b*$^{-/-}$ and *Fcgr2b*$^{-/-}$. *Sting*$^{gt/gt}$ mice at the age of 6-7 months were tested by real-time PCR (N=10-17 per group). The mRNA expressions of interferon-inducible genes shown in (A) *Isg15, (B) Mx1,(C) Irf3,(D) Irf5, and (E) Irf7*. Data show as mean ± SEM (*$p < 0.05$ and **$p<0.01$).

6. Decrease dendritic cells, plasmacytoid dendritic cells and effector T cells in the double-deficient mice

The splenocytes were analyzed from the mice at the age of 6-7 months to characterize the alteration of immune-phenotypes. The expansion of dendritic cells (CD11c+) and plasmacytoid dendritic cells (CD11c+PDCA+) in the Fcgr2b-/- mice diminished in the Fcgr2b-/-.Stinggt/gt mice

(Figure 6A-6B). Also, these data found that the reduction of T effector memory cells (CD3+CD4+CD62LloCD44hi), CD3+CD4+ICOS+ cells, and germinal center B cells (B220+GL7+) in the double-deficient mice (Figure 6C-6E). Besides, the mean fluorescence intensity of MHC-II (IA-b) on B cells significantly reduced in the double-deficient mice (Figure 15F). However, the expansion of plasma cells did not show the difference between single and double-deficient mice (Figure 6G).

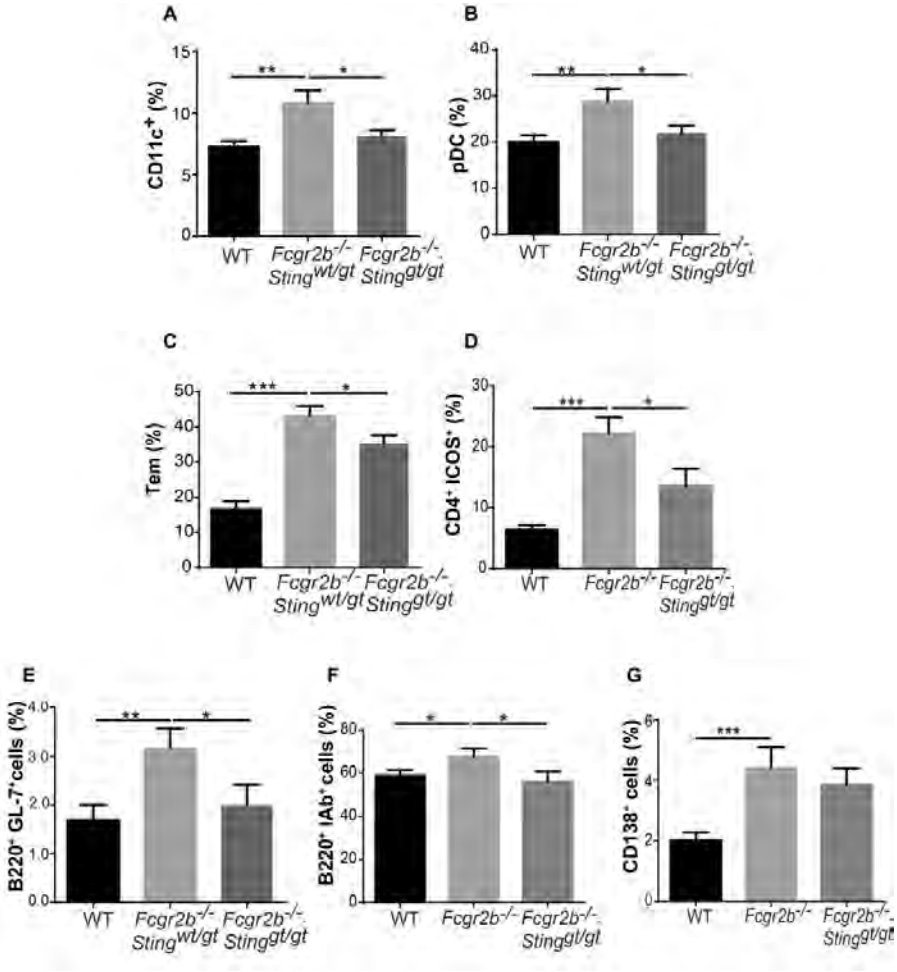

Figure 6 Decrease activated immune cells in the double deficient mice

(A-G) Flow cytometry analysis of splenocytes isolated from wild-type, Fcgr2b-/-, and Fcgr2b-/-. Stinggt/gt mice at the age of 6-7 months (N= 13-14 per group). Data shown in the percentage of (A) CD11c+, (B) plasmacytoid dendritic cells (pDC), (C) T effector memory (CD3+CD4+CD44hiCD62Llo), (D) CD3+CD4+ICOS+ cells, (E) B220+GL7+ cells and (G) CD138+ cells. Data show as mean ± SEM (*p < 0.05, **p<0.01 and ***p<0.001).

7. Sting activation increases the maturation of dendritic cells and cytokine production

In order to understand the immunological importance of Sting in lupus *Fcgr2b*-deficient mice, the flow cytometry was characterize the subsets of splenocytes from affected mice and their controls. The activated immune cells showed that the expansion of the DC in the spleen of the *Fcgr2b*$^{-/-}$ mice was Sting-dependent, and the cGAS/Sting pathway is important for DC activation. To investigate if the expansion of DC in the *Fcgr2b*$^{-/-}$ mice is directly mediated by Sting signaling, the bone-marrow derived dendritic cells (BMDC) were differentiated into immature DC and subsequently stimulated with Sting ligands (DMXAA), DMSO, and LPS (as a control) to assess if Sting played a role in DC maturation. The LPS control induced the immature DC to increase the expression of MHC-II (IA-b) and CD80, which suggested the phenotypes of mature DC, from both *Sting*-sufficient and *Sting*-deficient mice (Figure 7A and 7C). While the immature DC from wild-type and *Fcgr2b*$^{-/-}$ mice also showed the increasing percentage of IA-b^{+} and CD80^{+} DC cells after DMXAA stimulation, but the *Sting*-deficient mice did not develop these mature phenotypes (Figure 7B and 7D).

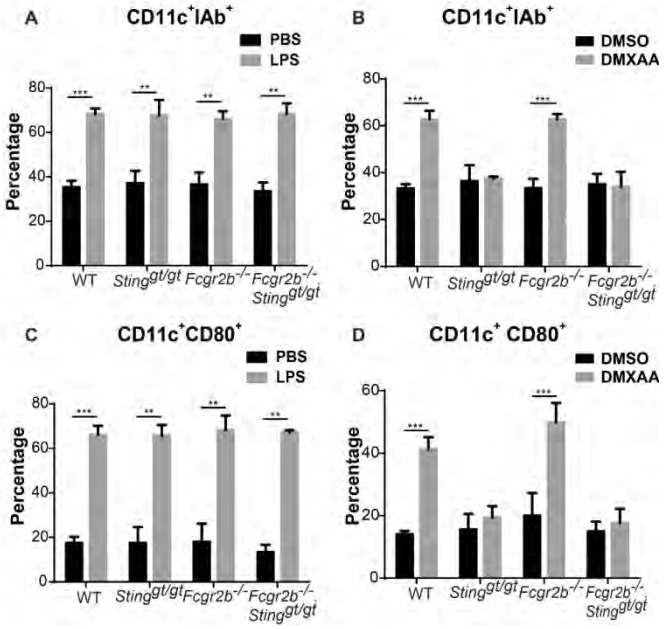

Figure 7 Sting activation increases the maturation of dendritic cells.

Bone marrows were isolated from wild-type, *Sting*$^{gt/gt}$, *Fcgr2b*$^{-/-}$ and *Fcgr2b*$^{-/-}$. *Sting*$^{gt/gt}$ mice at the age of 6-7 months. (A-D) IL-4 and GM-CSF differentiated bone marrow-derived dendritic cells (BMDC) for five days then immature BMDC were stimulated with LPS or DMXAA

for 24 hours. Flow cytometry analysis shows the percentage of (A-B) $CD11c^+$ IAb^+ cells and (C-D) $CD11c^+$ $CD80^+$ cells. Data show as mean ± SEM (N=3–5; *p < 0.05, **p<0.01 and ***p<0.001).

Furthermore, the supernatant from BMDC culture with DMXAA stimulation showed the increase in the concentration of IL-1α, IL-6, TNF-α, and MCP-1 in the wild-type and *Fcgr2b*$^{-/-}$ mice but not in Sting-deficient mice and double-deficient mice (Figure 8A-8D). These data suggested that Sting signaling pathway mediated DC maturation, activation stage, and cytokine production.

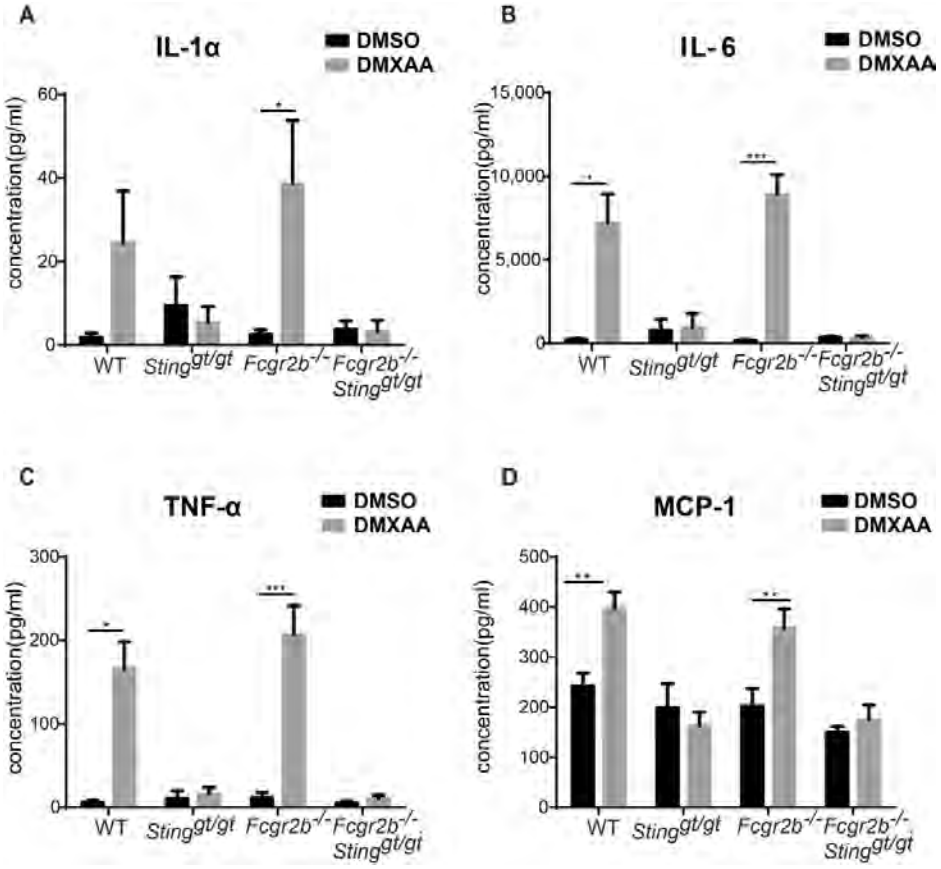

Figure 8 Sting-mediated signaling promotes cytokine production in DC.

(A-D) Supernatants were collected and analyzed after DMXAA stimulation for 24 hours. Cytometric bead array shows the levels of (A) IL-1α, (B) IL-6, (C) TNF-α and (D) MCP-1. Data show as mean ± SEM (N=3–5; *p < 0.05, **p<0.01 and ***p<0.001).

8. Sting signaling promotes the production of IFN-regulated proteins from BMDC

To better understand the function of Sting in DC, the quantitative proteomic analysis of Sting activated BMDC in the *Fcgr2b*$^{-/-}$ mice compared to the double-deficient mice were performed using a dimethyl labeling method. The Volcano plot showed the protein that highly expressed were interferon-regulated proteins (Figure 9). This finding may result from the increase of IFN-I production in the culture medium, which could upregulate the interferon-regulated proteins.

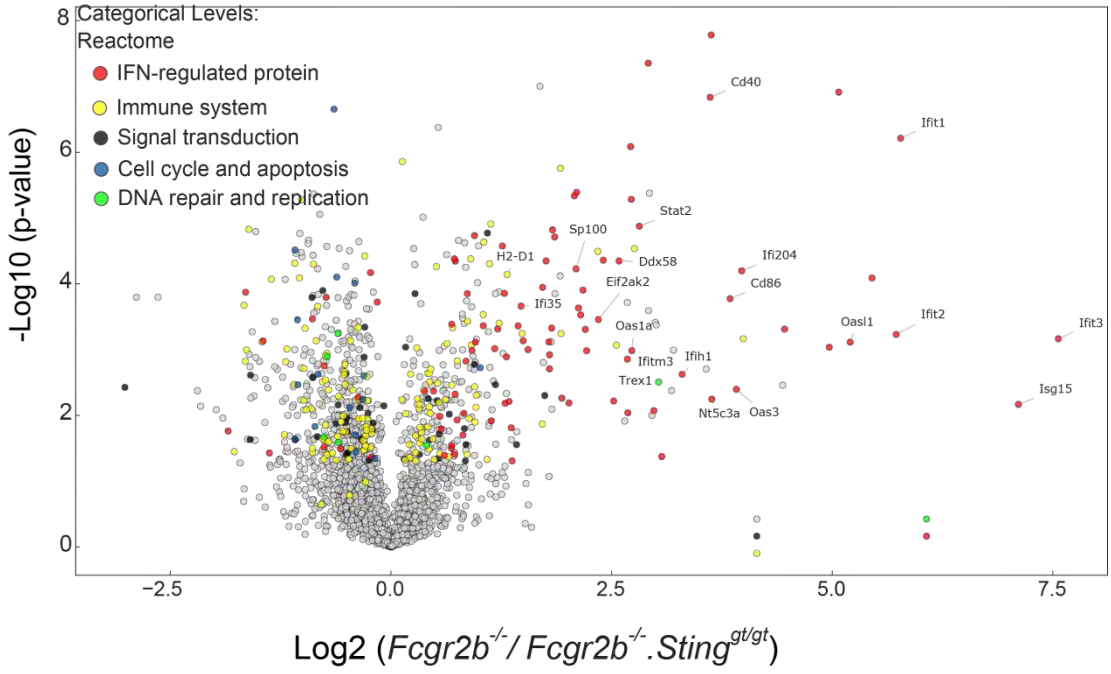

Figure 9 *Fcgr2b*$^{-/-}$ increase the expression of interferon-regulated proteins.

Volcano plot of protein expressions from proteomic analysis of DMXAA activated BMDC of *Fcgr2b*$^{-/-}$ and *Fcgr2b*$^{-/-}$. *Sting*$^{gt/gt}$ mice at the age of 6-7 months (N=4 per group).

9. Sting signaling promotes the differentiation of plasmacytoid dendritic cells (pDC)

The hypothesis of this study is Sting might promote the differentiation of pDC (the major producer of IFN-I). To confirm this hypothesis, the in vitro culture of BMDC with DMXAA and LPS (as a control) showed a significant increase in pDC and IFN-β production with DMXAA but not with LPS stimulation (Figure 10A-10D). Also, the results show the morphology of these cells by the imaging flow cytometry and found the pDC expressed CD80 and IA-b as well (Figure 10E - 10F).

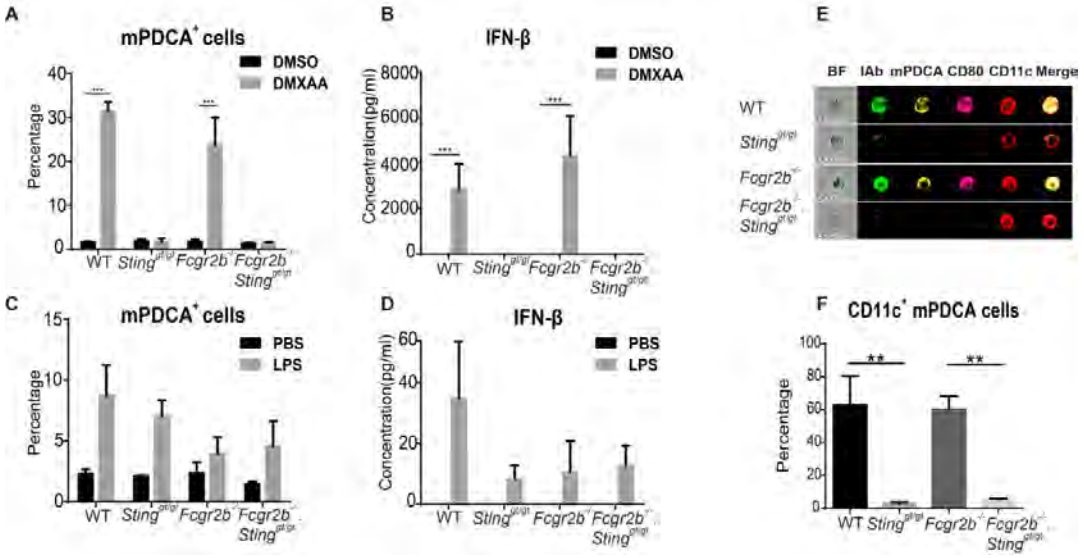

Figure 10 Sting activation promotes differentiation of plasmacytoid dendritic cells (pDC).

The percentage of pDC ($PDCA^+$ cells) after (A) DMXAA activation and (C) LPS activation for 24 hours (N = 3–4 per group). (B and D) The level of IFN-β from the culture supernatant of activated BMDC with (B) DMXAA and (C) LPS (N = 5 per group). (E and F) Imaging flow cytometry of DMXAA activated BMDC shows (E) the representative staining of IA-b (green), mPDCA (yellow), CD80 (pink), and CD11c (red) and (F) the percentage of $CD11c^+mPDCA^+$ cells (N= 3 mice per group). A representative of 3 experiments. Data show as mean± SEM (**p<0.01 and ***p<0.001).

10. Adoptive transfer of Sting expressing BMDC induce lupus development in the *Fcgr2b*$^{-/-}$*.Sting*$^{gt/gt}$ mice

The Sting signaling pathway activated the immature BMDC to differentiate into the mature DC and pDC which are capable of promoting T cell proliferation and producing the inflammatory cytokines. The proposed of this study is that Sting may induce the lupus disease by initially acting through the DC activation. To confirm this hypothesis, the adoptive transfers of Sting-activated BMDC into the double-deficient mice were performed. The anti-dsDNA significantly increased in the recipient mice who received *Sting*-sufficient BMDC compared to the non-recipient controls (Figure 11). The BMDC derived from Sting-sufficient mice (both WT and *Fcgr2b*$^{-/-}$) induced the recipients to produce a higher titer of anti-dsDNA than the ones from the double-deficient mice(Figure 11).

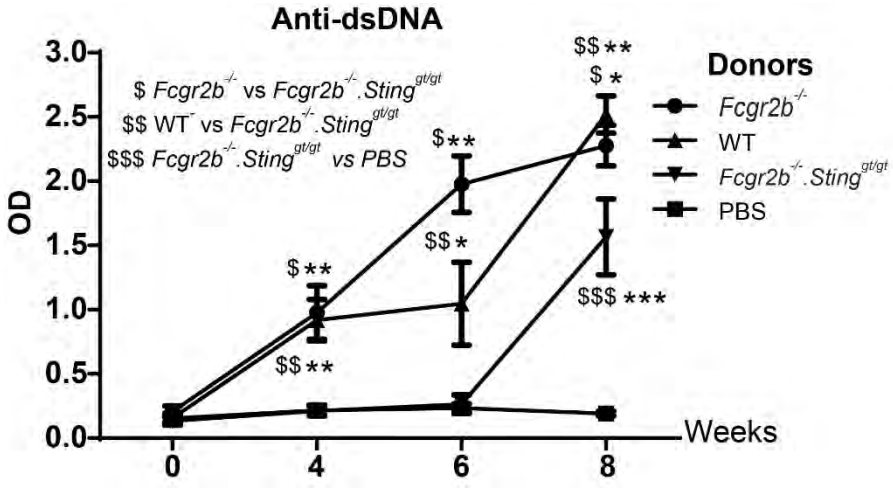

Figure 11 Increase of anti-dsDNA in the recipient of double-deficient mice.

DMXAA activated BMDC from *Fcgr2b*$^{-/-}$, WT, and *Fcgr2b*$^{-/-}$*.Sting*$^{gt/gt}$ were transferred into the recipient mice (*Fcgr2b*$^{-/-}$*. Sting*$^{gt/gt)}$. (A) The level of anti-dsDNA from the sera (1:100) measured by ELISA (N=5-10 per group). Data show as mean ± SEM (*p < 0.05, **p<0.01, and ***p<0.001).

In addition, the flow cytometry analysis of spleens from all groups of recipient mice showed the increase in the percentage of T effector memory (T_{em}), $CD4^+ICOS^+$, and germinal center B cells when compared with PBS injection group (Figure 12A-12C). However, the transfer of Sting-activated BMDC from the *Fcgr2b*$^{-/-}$ mice significantly induced the T effector memory (T_{em}), $CD4^+ICOS^+$, and germinal center B cells, but did not increase $CD138^+$ cells, $B220^+IAb^+$ cells (Figure 12D).

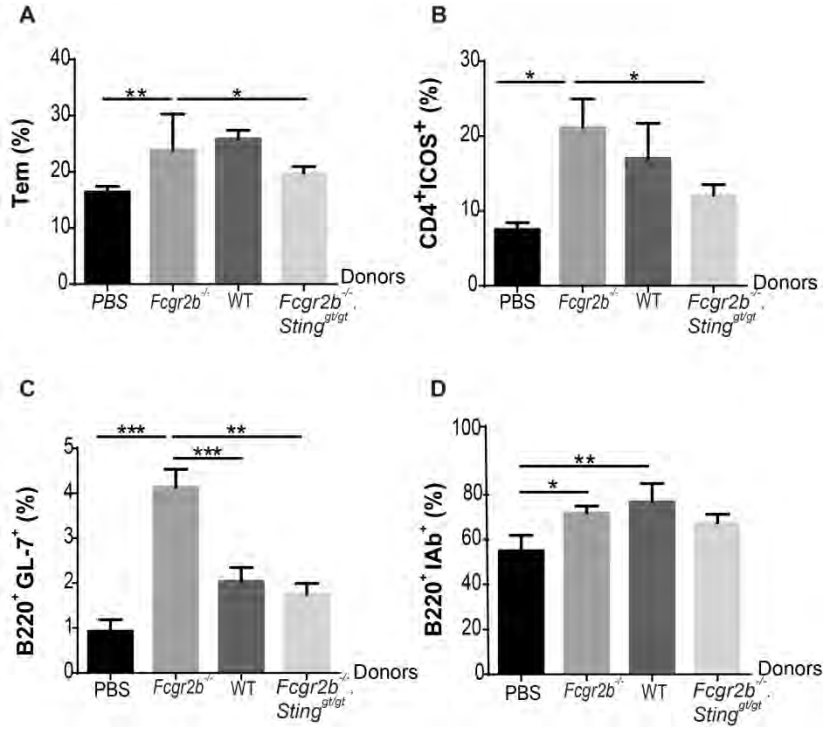

Figure 12 Increase of activated immune cells in recipient of double-deficient mice.

(A-E) Flow cytometry analysis of recipient splenocytes after BMDC transferred every 2 weeks for 4 times show the percentage of (A) effector T cells ($CD4^+CD44^{hi}CD62L^{lo}$), (B) $CD4^+ICOS^+$ cells, (C) $B220^+GL7^+$ cells and (D) $B220^+IAb^+$ cells (N=5-10 per group). Data show as mean ± SEM (*$p < 0.05$, **$p<0.01$, and ***$p<0.001$).

3. อภิปราย / วิจารณ์ (Discussion) ผลการทดลอง / ผลการวิจัยที่ได้ทั้งหมด (ทั้งที่เป็นและไม่เป็นไปตามสมมติฐานที่ตั้งไว้)

The survival of the 129/B6.*Fcgr2b*$^{-/-}$ mice depends on the autoantibody production and glomerulonephritis (55, 83) and the data from the previous study indicated that 6 months old of Fcgr2b$^{-/-}$ generated in 129 background mice showed the full lupus phenotypes (28). Moreover, it is found that Sting is required for the antibody production induced by cyclic-di-GMP in vitro (84). This study suggested that Sting facilitated the autoantibody production, inflammatory cell infiltration, and glomerulonephritis in the 129/B6.*Fcgr2b*$^{-/-}$ mice. Therefore, in the absent of Sting, resulting in improved the survival rate of 129/B6.*Fcgr2b*$^{-/-}$ mice.

Investigations by several studies conclude that the expression of interferon-inducible genes associated with SLE disease activity (85). This study detected the very high expression of a hundred of IFN inducible genes in the kidneys of 129/B6.*Fcgr2b*$^{-/-}$ mice by microarray that showed more severe pathology. The absence of Sting signaling in the *Fcgr2b*$^{-/-}$ mice partly decreased the expression of interferon-inducible genes in the kidney. This data suggested that other nucleic acid sensors may promote the type I interferon production or signaling leads to destructive of kidneys in the *Fcgr2b*$^{-/-}$ mice as well. The Sting-dependent lupus phenotypes do not mediate only through type-I interferon pathway.

Sting expresses and functions differentially depended on the cell types. The previous study reported that STING was a low expression in B cell from SLE patients and MRL/lpr mice. These finding may contribute to the pathogenesis of SLE by increasing the activation of the JAK1-STAT1 signaling indirectly by STING (86). Whereas, Sting signals coordinately with B cell receptor (BCR) signaling to promote antibody response (87). The results showed that the spontaneous germinal center B cells and MHC-II expression in the *Fcgr2b*$^{-/-}$ mice were *Sting*-dependent. However, plasma cell expansion was *Sting*-independent. This data suggested Sting may contribute to the autoantibody production through memory B cells.

Sting also activates T cells by treatment with Sting ligand (DMXAA) induced not only *Sting*-dependent expression of ISGs and type I IFN production but also mediated cell stress and death (88). Nevertheless, this study found that the increase of T effector memory (T_{em}) in the *Fcgr2b*$^{-/-}$ mice was Sting-dependent. The expansion of T_{em} may directly mediate through the interaction with antigen presenting cells, not directly via Sting signaling in T cells.

Sting agonist (DMXAA) treated mice show the increased expression of CD80, CD86, and MHC-II on DC and IFN-β production suggesting promotes the mature phenotypes of DC as the antigen-presenting cells (APC) which increase the expansion of T cells (89). This observation found the reduction of DC expansion in the *Fcgr2b*$^{-/-}$ mice, which depended on Sting signaling.

Depletion of pDC ameliorates the autoimmune phenotypes in BXSB lupus-prone mice and B6.Nba2 mice (96, 97). These data strongly suggested Sting involving in DC function both DC maturation and pDC differentiation. The adoptive transfer of *Sting* sufficient BMDC can induce autoantibody production regardless of *Fcgr2b* status. However, the absence of *Fcg2b* in the BMDC can accelerate the autoimmune phenotypes, including the immune complex deposition and inflammatory cell infiltration in the double-deficient recipient mice. Additionally, the adoptive transfers of *Sting sufficient* BMDC derived from Fcg2-/- mice increase the antibody production and activated immune cells but did not change the kidney pathology in wild type recipients.

Nevertheless, wild type recipient mice do not develop glomerulonephritis. The data implied that these wild type mice require lupus-susceptibility gene to progress of the disease (79). In summary, these data elaborated the vital function of Sting in the autoimmune *Fcgr2b*$^{-/-}$ lupus mouse model. The inhibition of STING signaling is a promising therapeutic target for SLE patients.

4. สรุปและเสนอแนะเกี่ยวกับการวิจัยในขั้นต่อไป ตลอดจนประโยชน์ในทางประยุกต์ของผลงานวิจัยที่ได้

129/B6.*Fcgr2b*$^{-/-}$ mice present the strong lupus phenotype

The *Fcgr2b*-deficient mice start to die at the age of 6 months, and the survival rates drop to 22.2 % by 12 months old, while the survival rates of double-deficient mice are 77.7 %. The effect of one allele of Sting to survival rates of Fcgr2b-deficient mice does not show a significant difference. This finding concludes that Sting increases survival rates and improves the lupus phenotypes in 129/B6.*Fcgr2b*$^{-/-}$ lupus mice model.

The activation of Sting pathway involved in the pathogenesis of SLE in lupus mice

In the absence of Sting, the lupus phenotypes of 129/B6.*Fcgr2b*-deficient mice were improved, including:

1. The kidney staining of Fcgr2b-deficient mice shows inflammatory cell infiltrations, enlarged glomeruli, and crescentic glomeruli, but double-deficient mice do not develop glomerulonephritis.

2. Antinuclear antibody (ANA) and anti-dsDNA production in the serum were decreases in the double-deficient mice. The results suggest that the high levels of autoantibodies are from the Sting-dependent.

3. Sting-mediated signaling induces type I interferon production and leads to the increase of interferon-inducible gene expression while the interferon signature genes in the kidneys were diminished in the double-deficient mice.

4. In order to understand the immunological importance of Sting in lupus *Fcgr2b*-deficient mice, the flow cytometry were characterized by the subsets of splenocytes from affected mice and their controls. The activated immune cells decrease in the percentage and numbers in the double-deficient mice, especially CD11c^{+}cells, plasmacytoid dendritic cells, and effector T cells. Also, B cells in the germinal center reduced in the double-deficient mice but not plasma cells.

The Sting-mediated pathway contribute to SLE via DNA sensor-mediated signaling in antigen presenting cells

The adoptive transfer of Sting-activated bone marrow-derived dendritic cells (BMDC) into the *Sting*-deficiency 129/B6.*Fcgr2b*$^{-/-}$ mice restored the lupus phenotypes. These data suggested that Sting signaling expressed in the dendritic cells induced the autoimmune development in the 129/B6.*Fcgr2b*$^{-/-}$ mice.

ส่วนประกอบตอนท้าย

1.บรรณานุกรม (Bibliography) ระบุรายชื่อเอกสารอ้างอิงโดยเรียงลำดับเอกสารอ้างอิงภาษาไทยก่อนแล้วตามด้วยเอกสารภาษาต่างประเทศ ทั้งนี้ให้เรียงตามลำดับ

1. 1. Muskardin TLW, Niewold TB. Type I interferon in rheumatic diseases. Nature Reviews Rheumatology. 2018;14(4):214.

2. Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proceedings of the National Academy of Sciences. 2003;100(5):2610-5.

3. Niewold TB, Clark DN, Salloum R, Poole BD. Interferon Alpha in Systemic Lupus Erythematosus. Journal of Biomedicine and Biotechnology. 2010;2010:8.

4. Lauwerys BR, Ducreux J, Houssiau FA. Type I interferon blockade in systemic lupus erythematosus: where do we stand? Rheumatology. 2013;53(8):1369-76.

5. Keating SE, Baran M, Bowie AG. Cytosolic DNA sensors regulating type I interferon induction. Trends in immunology. 2011;32(12):574-81.

6. Asselin-Paturel C, Brizard G, Chemin K, Boonstra A, O'Garra A, Vicari A, et al. Type I interferon dependence of plasmacytoid dendritic cell activation and migration. Journal of Experimental Medicine. 2005;201(7):1157-67.

7. Pisitkun P, Deane JA, Difilippantonio MJ, Tarasenko T, Satterthwaite AB, Bolland S. Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. Science. 2006;312(5780):1669-72.

8. Christensen SR, Shupe J, Nickerson K, Kashgarian M, Flavell RA, Shlomchik MJ. Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. Immunity. 2006;25(3):417-28.

9. Deane JA, Pisitkun P, Barrett RS, Feigenbaum L, Town T, Ward JM, et al. Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation. Immunity. 2007;27(5):801-10.

10. Sacre K, Criswell LA, McCune JM. Hydroxychloroquine is associated with impaired interferon-alpha and tumor necrosis factor-alpha production by plasmacytoid dendritic cells in systemic lupus erythematosus. Arthritis research & therapy. 2012;14(3):R155.

11. Yan N. Immune Diseases Associated with TREX1 and STING Dysfunction. Journal of Interferon & Cytokine Research. 2017;37(5):198-206.

12. Crow YJ, Hayward BE, Parmar R, Robins P, Leitch A, Ali M, et al. Mutations in the gene encoding the 3′-5′ DNA exonuclease TREX1 cause Aicardi-Goutieres syndrome at the AGS1 locus. Nature genetics. 2006;38(8):917.

13. Peschke K, Friebe F, Zimmermann N, Wahlicht T, Schumann T, Achleitner M, et al. Deregulated type I IFN response in TREX1-associated familial chilblain lupus. The Journal of investigative dermatology. 2014;134(5):1456.

14. Morita M, Stamp G, Robins P, Dulic A, Rosewell I, Hrivnak G, et al. Gene-targeted mice lacking the Trex1 (DNase III) 3′⟶ 5′ DNA exonuclease develop inflammatory myocarditis. Molecular and cellular biology. 2004;24(15):6719-27.

15. Stetson DB, Ko JS, Heidmann T, Medzhitov R. Trex1 prevents cell-intrinsic initiation of autoimmunity. Cell. 2008;134(4):587-98.

16. Ahn J, Gutman D, Saijo S, Barber GN. STING manifests self DNA-dependent inflammatory disease. Proceedings of the National Academy of Sciences. 2012;109(47):19386-91.

17. Burdette DL, Vance RE. STING and the innate immune response to nucleic acids in the cytosol. Nature immunology. 2013;14(1):19.

18. Cai X, Chiu Y-H, Chen ZJ. The cGAS-cGAMP-STING pathway of cytosolic DNA sensing and signaling. Molecular cell. 2014;54(2):289-96.

19. Ablasser A, Goldeck M, Cavlar T, Deimling T, Witte G, Röhl I, et al. cGAS produces a 2′-5′-linked cyclic dinucleotide second messenger that activates STING. Nature. 2013;498(7454):380.

20. Wu J, Sun L, Chen X, Du F, Shi H, Chen C, et al. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. Science. 2013;339(6121):826-30.

21. Gall A, Treuting P, Elkon KB, Loo Y-M, Gale M, Jr., Barber GN, et al. Autoimmunity initiates in nonhematopoietic cells and progresses via lymphocytes in an interferon-dependent autoimmune disease. Immunity. 2012;36(1):120-31.

22. Perry D, Sang A, Yin Y, Zheng Y-Y, Morel L. Murine Models of Systemic Lupus Erythematosus. Journal of Biomedicine and Biotechnology. 2011;2011.

23. Murphy ED, Roths JB. A y chromosome associated factor in strain bxsb producing accelerated autoimmunity and lymphoproliferation. Arthritis & Rheumatism. 1979;22(11):1188-94.

24. Nickerson KM, Christensen SR, Shupe J, Kashgarian M, Kim D, Elkon K, et al. TLR9 Regulates TLR7- and MyD88-Dependent Autoantibody Production and Disease in a Murine Model of Lupus. The Journal of Immunology. 2010;184(4):1840.

25. Sharma S, Campbell AM, Chan J, Schattgen SA, Orlowski GM, Nayar R, et al. Suppression of systemic autoimmunity by the innate immune adaptor STING. Proceedings of the National Academy of Sciences. 2015;112(7):E710.

26. Hron JD, Peng SL. Type I IFN Protects Against Murine Lupus. The Journal of Immunology. 2004;173(3):2134.

27. Bolland S, Ravetch JV. Spontaneous Autoimmune Disease in FcγRIIB-Deficient Mice Results from Strain-Specific Epistasis. Immunity. 2000;13(2):277-85.

28. Boross P, Arandhara VL, Martin-Ramirez J, Santiago-Raber M-L, Carlucci F, Flierman R, et al. The Inhibiting Fc Receptor for IgG, FcγRIIB, Is a Modifier of Autoimmune Susceptibility. The Journal of Immunology. 2011;187(3):1304.

29. Choubey D. Interferon-inducible Ifi200-family genes as modifiers of lupus susceptibility. Immunology Letters. 2012;147(1):10-7.

30. Sato-Hayashizaki A, Ohtsuji M, Lin Q, Hou R, Ohtsuji N, Nishikawa K, et al. Presumptive role of 129 strain–derived Sle16 locus in rheumatoid arthritis in a new mouse model with Fcγ receptor type IIb–deficient C57BL/6 genetic background. Arthritis & Rheumatism. 2011;63(10):2930-8.

31. Rozzo SJ, Allard JD, Choubey D, Vyse TJ, Izui S, Peltz G, et al. Evidence for an Interferon-Inducible Gene, Ifi202, in the Susceptibility to Systemic Lupus. Immunity. 2001;15(3):435-43.

32. Almine JF, O'Hare CAJ, Dunphy G, Haga IR, Naik RJ, Atrih A, et al. IFI16 and cGAS cooperate in the activation of STING during DNA sensing in human keratinocytes. Nature Communications. 2017;8:14392.

33. Crispin JC, Hedrich CM, Tsokos GC. Gene-function studies in systemic lupus erythematosus. Nat Rev Rheumatol. 2013;9(8):476-84.

34. Bolland S, Yim Y-S, Tus K, Wakeland EK, Ravetch JV. Genetic Modifiers of Systemic Lupus Erythematosus in Fcγ RIIB^{−/−} Mice. The Journal of Experimental Medicine. 2002;195(9):1167.

35. Kyogoku C, Dijstelbloem HM, Tsuchiya N, Hatta Y, Kato H, Yamaguchi A, et al. Fcγ receptor gene polymorphisms in Japanese patients with systemic lupus erythematosus: Contribution of FCGR2B to genetic susceptibility. Arthritis & Rheumatism. 2002;46(5):1242-54.

36. Szczerba BM, Rybakowska PD, Dey P, Payerhin KM, Peck AB, Bagavant H, et al. Type I Interferon Receptor Deficiency Prevents Murine Sjögren's Syndrome. Journal of Dental Research. 2013;92(5):444-9.

37. Perl A. Pathogenic mechanisms in systemic lupus erythematosus. Autoimmunity. 2010;43(1):1-6.

38. Autoimmune Disease: Mechanisms. eLS.

39. Smyk D, Rigopoulou EI, Baum H, Burroughs AK, Vergani D, Bogdanos DP. Autoimmunity and Environment: Am I at risk? Clinical Reviews in Allergy & Immunology. 2012;42(2):199-212.

40. Crampton SP, Morawski PA, Bolland S. Linking susceptibility genes and pathogenesis mechanisms using mouse models of systemic lupus erythematosus. Disease Models & Mechanisms. 2014;7(9):1033.

41. Jakes RW, Bae S-C, Louthrenoo W, Mok C-C, Navarra SV, Kwon N. Systematic review of the epidemiology of systemic lupus erythematosus in the Asia-Pacific region: Prevalence, incidence, clinical features, and mortality. Arthritis Care & Research. 2012;64(2):159-68.

42. Tsuchiya N, Kyogoku C. Role of Fcγ receptor IIb polymorphism in the genetic background of systemic lupus erythematosus: Insights from Asia. Autoimmunity. 2005;38(5):347-52.

43. Krishnamurthy S, Mahadevan S. Systemic Lupus Erythematosus: Recent Concepts in Genomics, Pathogenetic Mechanisms, and Therapies. ISRN Immunology. 2011;2011:7.

44. Ohl K, Tenbrock K. Inflammatory cytokines in systemic lupus erythematosus. Journal of Biomedicine and Biotechnology. 2011;2011.

45. Kim J-M, Park S-H, Kim H-Y, Kwok S-K. A Plasmacytoid Dendritic Cells-Type I Interferon Axis Is Critically Implicated in the Pathogenesis of Systemic Lupus Erythematosus. International Journal of Molecular Sciences. 2015;16(6):14158-70.

46. Niewold TB. Interferon alpha as a primary pathogenic factor in human lupus. J Interferon Cytokine Res. 2011;31(12):887-92.

47. Joo H, Coquery C, Xue Y, Gayet I, Dillon SR, Punaro M, et al. Serum from patients with SLE instructs monocytes to promote IgG and IgA plasmablast differentiation. The Journal of Experimental Medicine. 2012;209(7):1335-48.

48. Banchereau J, Pascual V. Type I Interferon in Systemic Lupus Erythematosus and Other Autoimmune Diseases. Immunity. 2006;25(3):383-92.

49. Nacionales DC, Kelly-Scumpia KM, Lee PY, Weinstein JS, Lyons R, Sobel E, et al. DEFICIENCY OF THE TYPE I INTERFERON RECEPTOR PROTECTS MICE FROM EXPERIMENTAL LUPUS. Arthritis and rheumatism. 2007;56(11):3770-83.

50. Obermoser G, Pascual V. The interferon-α signature of systemic lupus erythematosus. Lupus. 2010;19(9):1012-9.

51. Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proceedings of the National Academy of Sciences. 2003;100(5):2610-5.

52. Ronnblom L, Elkon KB. Cytokines as therapeutic targets in SLE. Nat Rev Rheumatol. 2010;6(6):339-47.

53. Crampton SP, Morawski PA, Bolland S. Linking susceptibility genes and pathogenesis mechanisms using mouse models of systemic lupus erythematosus. Disease Models & Mechanisms. 2014;7(9):1033-46.

54. Kirou KA, Gkrouzman E. Anti-interferon alpha treatment in SLE. Clinical Immunology. 2013;148(3):303-12.

55. Bolland S, Yim Y-S, Tus K, Wakeland EK, Ravetch JV. Genetic Modifiers of Systemic Lupus Erythematosus in FcγRIIB(−/−) Mice. The Journal of Experimental Medicine. 2002;195(9):1167-74.

56. Park H, Sheen DH, Lim MK, Shim SC. Animal Models in Systemic Lupus Erythematosus. J Rheum Dis. 2012;19(4):173-88.

57. Bygrave AE, Rose KL, Cortes-Hernandez J, Warren J, Rigby RJ, Cook HT, et al. Spontaneous Autoimmunity in 129 and C57BL/6 Mice—Implications for Autoimmunity Described in Gene-Targeted Mice. PLOS Biology. 2004;2(8):e243.

58. Smith KGC, Clatworthy MR. FcgammaRIIB in autoimmunity and infection: evolutionary and therapeutic implications. Nature reviews Immunology. 2010;10(5):328-43.

59. Niederer HA, Clatworthy MR, Willcocks LC, Smith KGC. FcγRIIB, FcγRIIIB, and systemic lupus erythematosus. Annals of the New York Academy of Sciences. 2010;1183(1):69-88.

60. Nimmerjahn F, Ravetch JV. Fcγ Receptors: Old Friends and New Family Members. Immunity. 2006;24(1):19-28.

61. Takai T. Roles of Fc receptors in autoimmunity. Nature Reviews Immunology. 2002;2(8):580-92.

62. Malbec O, Fong DC, Turner M, Tybulewicz VLJ, Cambier JC, Fridman WH, et al. Fcε Receptor I-Associated lyn-Dependent Phosphorylation of Fcγ Receptor IIB During Negative Regulation of Mast Cell Activation. The Journal of Immunology. 1998;160(4):1647.

63. Leiss H, Niederreiter B, Bandur T, Schwarzecker B, Blüml S, Steiner G, et al. Pristane-induced lupus as a model of human lupus arthritis: evolvement of autoantibodies, internal organ and joint inflammation. Lupus. 2013;22(8):778-92.

64. Reeves WH, Lee PY, Weinstein JS, Satoh M, Lu L. Induction of autoimmunity by pristane and other naturally occurring hydrocarbons. Trends in Immunology. 2009;30(9):455-64.

65. Zhuang H, Szeto C, Han S, Yang L, Reeves WH. Animal Models of Interferon Signature Positive Lupus. Frontiers in Immunology. 2015;6(291).

66. Barrat FJ, Elkon KB, Fitzgerald KA. Importance of Nucleic Acid Recognition in Inflammation and Autoimmunity. Annual Review of Medicine. 2016;67(1):323-36.

67. Härtlova A, Erttmann SF, Raffi FAM, Schmalz AM, Resch U, Anugula S, et al. DNA Damage Primes the Type I Interferon System via the Cytosolic DNA Sensor STING to Promote Anti-Microbial Innate Immunity. Immunity. 2015;42(2):332-43.

68. Ishikawa H, Ma Z, Barber GN. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature. 2009;461(7265):788-92.

69. Bode C, Fox M, Tewary P, Steinhagen A, Ellerkmann RK, Klinman D, et al. Human plasmacytoid dendritic cells elicit a Type I Interferon response by sensing DNA via the cGAS-STING signaling pathway. European Journal of Immunology. 2016;46(7):1615-21.

70. Jeremiah N, Neven B, Gentili M, Callebaut I, Maschalidi S, Stolzenberg M-C, et al. Inherited STING-activating mutation underlies a familial inflammatory syndrome with lupus-like manifestations. The Journal of Clinical Investigation. 2014;124(12):5516-20.

71. Liu Y, Jesus AA, Marrero B, Yang D, Ramsey SE, Montealegre Sanchez GA, et al. Activated STING in a Vascular and Pulmonary Syndrome. New England Journal of Medicine. 2014;371(6):507-18.

72. Chan O, Madaio MP, Shlomchik MJ. The Roles of B Cells in MRL/lpr Murine Lupus. Annals of the New York Academy of Sciences. 1997;815(1):75-82.

73. Makjaroen J, Somparn P, Hodge K, Poomipak W, Hirankarn N, Pisitkun T. Comprehensive Proteomics Identification of IFN-λ3-regulated Antiviral Proteins in HBV-transfected Cells. Molecular & Cellular Proteomics. 2018;17(11):2197.

74. Sauer J-D, Sotelo-Troha K, von Moltke J, Monroe KM, Rae CS, Brubaker SW, et al. The N-ethyl-N-nitrosourea-induced Goldenticket mouse mutant reveals an essential function of Sting in the in vivo interferon response to Listeria monocytogenes and cyclic dinucleotides. Infect Immun. 2011;79(2):688-94.

75. Marinho FV, Benmerzoug S, Rose S, Campos PC, Marques JT, Bafica A, et al. The cGAS/STING Pathway Is Important for Dendritic Cell Activation but Is Not Essential to Induce Protective Immunity against Mycobacterium tuberculosis Infection. J Innate Immun. 2018;10(3):239-52.

76. Chu CL, Lowell CA. The Lyn tyrosine kinase differentially regulates dendritic cell generation and maturation. J Immunol. 2005;175(5):2880-9.

77. Dallari S, Macal M, Loureiro ME, Jo Y, Swanson L, Hesser C, et al. Src family kinases Fyn and Lyn are constitutively activated and mediate plasmacytoid dendritic cell responses. Nature Communications. 2017;8:14830.

78. Chu C-L, Lowell CA. The Lyn Tyrosine Kinase Differentially Regulates Dendritic Cell Generation and Maturation. The Journal of Immunology. 2005;175(5):2880.

79. Ichii O, Kamikawa A, Otsuka S, Hashimoto Y, Sasaki N, Endoh D, et al. Overexpression of interferon-activated gene 202 (Ifi202) correlates with the progression of autoimmune glomerulonephritis associated with the MRL chromosome 1. Lupus. 2010;19(8):897-905.

80. Panchanathan R, Liu H, Xin D, Choubey D. Identification of a negative feedback loop between cyclic di-GMP-induced levels of IFI16 and p202 cytosolic DNA sensors and STING. Innate Immunity. 2013;20(7):751-9.

81. Burdette DL, Vance RE. STING and the innate immune response to nucleic acids in the cytosol. Nature Immunology. 2012;14:19.

82. Panchanathan R, Shen H, Duan X, Rathinam VAK, Erickson LD, Fitzgerald KA, et al. Aim2 Deficiency in Mice Suppresses the Expression of the Inhibitory Fcγ Receptor (FcγRIIB) through the Induction of the IFN-Inducible p202, a Lupus Susceptibility Protein. The Journal of Immunology. 2011;186(12):6762.

83. Pisitkun P, Ha H-L, Wang H, Claudio E, Tivy Caitlyn C, Zhou H, et al. Interleukin-17 Cytokines Are Critical in Development of Fatal Lupus Glomerulonephritis. Immunity. 2012;37(6):1104-15.

84. Walker MM, Crute BW, Cambier JC, Getahun A. B Cell–Intrinsic STING Signaling Triggers Cell Activation, Synergizes with B Cell Receptor Signals, and Promotes Antibody Responses. The Journal of Immunology. 2018;201(9):2641.

85. Feng X, Wu H, Grossman JM, Hanvivadhanakul P, FitzGerald JD, Park GS, et al. Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. Arthritis & Rheumatism. 2006;54(9):2951-62.

86. Dong G, You M, Ding L, Fan H, Liu F, Ren D, et al. STING negatively regulates double-stranded DNA-activated JAK1-STAT1 signaling via SHP-1/2 in B cells2015.

87. Walker MM, Crute BW, Cambier JC, Getahun A. B Cell–Intrinsic STING Signaling Triggers Cell Activation, Synergizes with B Cell Receptor Signals, and Promotes Antibody Responses. The Journal of Immunology. 2018:ji1701405.

88. Larkin B, Ilyukha V, Sorokin M, Buzdin A, Vannier E, Poltorak A. Cutting Edge: Activation of STING in T Cells Induces Type I IFN Responses and Cell Death. Journal of immunology (Baltimore, Md : 1950). 2017;199(2):397-402.

89. Curran E, Chen X, Corrales L, Kline DE, Dubensky TW, Jr., Duttagupta P, et al. STING Pathway Activation Stimulates Potent Immunity against Acute Myeloid Leukemia. Cell reports. 2016;15(11):2357-66.

90. Woo S-R, Fuertes MB, Corrales L, Spranger S, Furdyna MJ, Leung MYK, et al. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. Immunity. 2014;41(5):830-42.

91. Tanaka Y, Chen ZJ. STING Specifies IRF3 Phosphorylation by TBK1 in the Cytosolic DNA Signaling Pathway. Science Signaling. 2012;5(214):ra20.

92. Zhong B, Yang Y, Li S, Wang Y-Y, Li Y, Diao F, et al. The Adaptor Protein MITA Links Virus-Sensing Receptors to IRF3 Transcription Factor Activation. Immunity. 2008;29(4):538-50.

93. Aiba Y, Kameyama M, Yamazaki T, Tedder TF, Kurosaki T. Regulation of B-cell development by BCAP and CD19 through their binding to phosphoinositide 3-kinase. Blood. 2008;111(3):1497-503.

94. Sutton P, Borgia JA, Bonomi P, Plate JM. Lyn, a Src family kinase, regulates activation of epidermal growth factor receptors in lung adenocarcinoma cells. Mol Cancer. 2013;12:76.

95. Liu B, Wang C, Chen P, Wang L, Cheng Y. RACK1 promotes radiation resistance in esophageal cancer via regulating AKT pathway and Bcl-2 expression. Biochem Biophys Res Commun. 2017;491(3):622-8.

96. Davison LM, Jørgensen TN. Sialic Acid–Binding Immunoglobulin-Type Lectin H–Positive Plasmacytoid Dendritic Cells Drive Spontaneous Lupus-like Disease Development in B6.Nba2 Mice. Arthritis & Rheumatology. 2015;67(4):1012-22.

97. Rowland SL, Riggs JM, Gilfillan S, Bugatti M, Vermi W, Kolbeck R, et al. Early, transient depletion of plasmacytoid dendritic cells ameliorates autoimmunity in a lupus model. The Journal of Experimental Medicine. 2014;211(10):1977.

2. ภาคผนวก (Appendix) ถ้ามี

3. ประวัตินักวิจัยและคณะ พร้อมหน่วยงานสังกัด

ส่วน ค : ประวัติคณะผู้วิจัย

1. หัวหน้าโครงการ

1. ชื่อ - นามสกุล (ภาษาไทย) นาย ไตรรักษ์ พิสิษฐ์กุล

ชื่อ - นามสกุล (ภาษาอังกฤษ) Mr. Trairak Pisitkun

2. เลขหมายบัตรประจำตัวประชาชน 4100700009959

ตำแหน่งปัจจุบัน อาจารย์

เงินเดือน 65,000 (บาท)

เวลาที่ใช้ทำวิจัย 40 (ชั่วโมง : สัปดาห์)

3. ฝ่ายวิจัย คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

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4. ประวัติการศึกษา

2003-2008 Postdoctoral Fellow, Epithelial Systems Biology Laboratory (ESBL), NHLBI, National Institutes of Health, Maryland, USA

2002 Board of Clinical Nephrology, Thailand

2002 Master of Science, Chulalongkorn University, Thailand

1998 Board of Internal Medicine, Thailand

1994 M.D. (First Class Honors), Mahidol University, Thailand

1992 Bachelor of Science, Mahidol University, Thailand

5. สาขาวิชาการที่มีความชำนาญพิเศษ Systems Biology

6. ประสบการณ์ที่เกี่ยวข้องกับการบริหารงานวิจัยทั้งภายในและภายนอกประเทศ โดยระบุสถานภาพในการทำการวิจัยว่าเป็นผู้อำนวยการแผนงานวิจัย หัวหน้าโครงการวิจัย หรือผู้ร่วมวิจัยในแต่ละผลงานวิจัย

7 ผู้อำนวยการแผนงานวิจัย : ชื่อแผนงานวิจัย -

7.1 หัวหน้าโครงการวิจัย : ชื่อโครงการวิจัย Systems biology of aberrant targeting and polarization of membrane proteins in disease

7.2 งานวิจัยที่ทำเสร็จแล้ว : ชื่อผลงานวิจัย ปีที่พิมพ์ การเผยแพร่ และแหล่งทุน (อาจมากกว่า 1 เรื่อง)

1. Sandoval PC, Slentz DH, Pisitkun T, Saeed F, Hoffert JD, Knepper MA. Proteome-wide measurement of protein half-lives and translation rates in vasopressin-sensitive collecting duct cells. J Am Soc Nephrol. 2013 manuscript in press

แหล่งทุน National Institutes of Health, Maryland, USA

2. Yu P, Pisitkun T, Wang G, Wang R, Katagiri Y, Gucek M, Knepper MA, Geller HM. Global analysis of neuronal phosphoproteome regulation by chondroitin sulfate proteoglycans. PLoS One. 2013;8(3):e59285. doi: 10.1371/journal.pone.0059285. Epub 2013 Mar 18.

แหล่งทุน National Institutes of Health, Maryland, USA

3. Jensen TB, Pisitkun T, Hoffert JD, Jensen UB, Fenton RA, Praetorius HA, Knepper MA, Praetorius J. Assessment of the Effect of 24-Hour Aldosterone Administration on Protein Abundance in Fluorescence-Sorted Mouse Distal Renal Tubules by Mass Spectrometry. Nephron Physiol. 2013 Feb 14;121(3-4):p9-p15. [Epub ahead of print]

แหล่งทุน Aarhus University, Denmark

4. Saeed F, Hoffert JD, Pisitkun T, Knepper MA. High performance phosphorylation site assignment algorithm for mass spectrometry data using multicore systems. ACM-BCB. 2012;667-672. DOI=10.1145/2382936.2383056 http://doi.acm.org/10.1145/2382936.2383056

แหล่งทุน National Institutes of Health, Maryland, USA

5. Saeed F, Pisitkun T, Knepper MA, Hoffert JD. An Efficient Algorithm for Clustering of Large-Scale Mass Spectrometry Data. Proceedings (IEEE Int Conf Bioinformatics Biomed). 2012 Oct 4:1-4.

แหล่งทุน National Institutes of Health, Maryland, USA

6. Miller RL, Sandoval PC, Pisitkun T, Knepper MA, Hoffert JD. Vasopressin inhibits apoptosis in renal collecting duct cells. Am J Physiol Renal Physiol. 2012 Nov 7. [Epub ahead of print]

แหล่งทุน National Institutes of Health, Maryland, USA

7. Zhao B, Pisitkun T, Hoffert JD, Knepper MA, Saeed F. CPhos: a program to calculate and visualize evolutionarily conserved functional phosphorylation sites. Proteomics. 2012 Sep 24. doi: 10.1002/pmic.201200189. [Epub ahead of print]

แหล่งทุน National Institutes of Health, Maryland, USA

8. Saeed F, Pisitkun T, Hoffert JD, Wang G, Gucek M, Knepper MA. An Efficient Dynamic Programming Algorithm for Phosphorylation Site Assignment of Large-Scale Mass Spectrometry Data. Proceedings (IEEE Int Conf Bioinformatics Biomed). 2012 Oct 4:618-625.

แหล่งทุน National Institutes of Health, Maryland, USA

9. Bolger SJ, Gonzales Hurtado PA, Hoffert JD, Saeed F, Pisitkun T, Knepper MA. Quantitative Phosphoproteomics in Nuclei of Vasopressin-Sensitive Renal Collecting Duct Cells. Am J Physiol Cell Physiol. 2012 Sep 19. [Epub ahead of print]

แหล่งทุน National Institutes of Health, Maryland, USA

10. Douglass J, Gunaratne R, Bradford D, Saeed F, Hoffert JD, Steinbach PJ, Knepper MA, Pisitkun T. Identifying Protein Kinase Target Preferences Using Mass Spectrometry. Am J Physiol Cell Physiol. 2012 Oct;303(7):C715-27.

แหล่งทุน National Institutes of Health, Maryland, USA

11. Vilbig RL, Sarkar A, Zischkau J, Knepper MA, Pisitkun T. An online tool for calculation of free-energy balance for the renal inner medulla. Am J Physiol Renal Physiol. 2012 Aug;303(3):F366-72.

แหล่งทุน National Institutes of Health, Maryland, USA

12. Pisitkun T, Gandolfo MT, Das S, Knepper MA, Bagnasco SM. Application of systems biology principles to protein biomarker discovery: Urinary exosomal proteome in renal transplantation. Proteomics Clin Appl. 2012 Jun;6(5-6):268-78.

แหล่งทุน National Institutes of Health, Maryland, USA

13. Schenk LK, Bolger SJ, Luginbuhl K, Gonzales PA, Rinschen MM, Yu MJ, Hoffert JD, Pisitkun T, Knepper MA. Quantitative proteomics identifies vasopressin-responsive nuclear proteins in collecting duct cells. J Am Soc Nephrol. 2012 Jun;23(6):1008-18.

แหล่งทุน National Institutes of Health, Maryland, USA

14. Hoffert JD, Pisitkun T, Song JH, Saeed F, Knepper MA. Dynamics of the G protein-coupled vasopressin V2 receptor signaling network revealed by quantitative phosphoproteomics. Mol Cell Proteomics. 2012 Feb;11(2):M111.014613.

แหล่งทุน National Institutes of Health, Maryland, USA

15. Pisitkun T, Hoffert JD, Saeed F, Knepper MA. NHLBI-AbDesigner: an online tool for design of peptide-directed antibodies. Am J Physiol Cell Physiol. 2012 Jan;302(1):C154-64.

แหล่งทุน National Institutes of Health, Maryland, USA

16. Zhao B, Knepper MA, Chou CL, Pisitkun T. Large-scale phosphotyrosine proteomic profiling of rat renal collecting duct epithelium reveals predominance of proteins involved in cell polarity determination. Am J Physiol Cell Physiol. 2012 Jan;302(1):C27-45.

แหล่งทุน National Institutes of Health, Maryland, USA

17. Hoffert JD, Pisitkun T, Miller RL. Conditional Allele Mouse Planner (CAMP): software to facilitate the planning and design of breeding strategies involving mice with conditional alleles. Transgenic Res. 2011 Aug 26. [Epub ahead of print]

แหล่งทุน National Institutes of Health, Maryland, USA

18. Saeed F, Pisitkun T, Knepper MA, Hoffert JD. Mining temporal patterns from iTRAQ mass spectrometry (LC-MS/MS) data. The Proceedings of the ISCA 3rd International Conference on Bioinformatics and Computational Biology (BiCoB). 2011 March:1:152-9. (ISBN: 978-1-880843-81-9)

แหล่งทุน National Institutes of Health, Maryland, USA

19. Feric M, Zhao B, Hoffert JD, Pisitkun T, Knepper MA. Large-scale phosphoproteomic analysis of membrane proteins in renal proximal and distal tubule. Am J Physiol Cell Physiol. 2011 Apr;300(4):C755-70.

แหล่งทุน National Institutes of Health, Maryland, USA

20. Khositseth S, Pisitkun T, Slentz DH, Wang G, Hoffert JD, Knepper MA, Yu MJ. Quantitative protein and mRNA profiling shows selective post-transcriptional control of protein expression by vasopressin in kidney cells. Mol Cell Proteomics. 2011 Jan;10(1):M110.004036.

แหล่งทุน National Institutes of Health, Maryland, USA

21. Gunaratne R, Braucht DW, Rinschen MM, Chou CL, Hoffert JD, Pisitkun T, Knepper MA. Quantitative phosphoproteomic analysis reveals cAMP/vasopressin-dependent signaling pathways in native renal thick ascending limb cells. Proc Natl Acad Sci U S A. 2010 Aug 31;107(35):15653-8.

แหล่งทุน National Institutes of Health, Maryland, USA

22. Hwang S, Chou CL, Yu MJ, Rinschen MM, Gunaratne R, Pisitkun T, Hoffert JD, Knepper MA. Vasopressin increases phosphorylation of Ser84 and Ser486 in Slc14a2 collecting duct urea transporters. Am J Physiol Renal Physiol. 2010 Sep;299(3):F559-67.

แหล่งทุน National Institutes of Health, Maryland, USA

23. Pisitkun T, Da Silva N, Belleannée C, Miller LR, Nelson R, Knepper MA, Brown D, Breton S. Proteomic analysis of V-ATPase-rich cells harvested from the kidney and epididymis by fluorescence activated cell sorting. Am J Physiol Cell Physiol. 2010 Jun;298(6):C1326-42.

แหล่งทุน National Institutes of Health, Maryland, USA

24. Rinschen MM, Yu MJ, Wang G, Boja ES, Hoffert JD, Pisitkun T, Knepper MA. Quantitative phosphoproteomic analysis reveals vasopressin V2-receptor-dependent signaling pathways in renal collecting duct cells. Proc Natl Acad Sci U S A. 2010 Feb 23;107(8):3882-7.

แหล่งทุน National Institutes of Health, Maryland, USA

25. Fernández-Llama P, Khositseth S, Gonzales PA, Star RA, Pisitkun T, Knepper MA. Tamm-Horsfall protein and urinary exosome isolation. Kidney Int. 2010 Apr;77(8):736-42.

แหล่งทุน National Institutes of Health, Maryland, USA

26. Xie L, Hoffert JD, Chou CL, Yu MJ, Pisitkun T, Knepper MA, Fenton RA. Quantitative analysis of aquaporin-2 phosphorylation. Am J Physiol Renal Physiol. 2010 Apr;298(4):F1018-23.

แหล่งทุน National Institutes of Health, Maryland, USA

27. Bansal AD, Hoffert JD, Pisitkun T, Hwang S, Chou CL, Boja ES, Wang G, Knepper MA. Phosphoproteomic profiling reveals vasopressin-regulated phosphorylation sites in collecting duct. J Am Soc Nephrol. 2010 Feb;21(2):303-15.

แหล่งทุน National Institutes of Health, Maryland, USA

28. Tchapyjnikov D, Li Y, Pisitkun T, Hoffert JD, Yu MJ, Knepper MA. Proteomic profiling of nuclei from native renal inner medullary collecting duct cells using LC-MS/MS. Physiol Genomics. 2010 Feb 4;40(3):167-83.

แหล่งทุน National Institutes of Health, Maryland, USA

29. Zwang NA, Hoffert JD, Pisitkun T, Moeller HB, Fenton RA, Knepper MA. Identification of phosphorylation-dependent binding partners of aquaporin-2 using protein mass spectrometry. J Proteome Res. 2009 Mar;8(3):1540-54.

แหล่งทุน National Institutes of Health, Maryland, USA

30. Yu MJ, Miller RL, Uawithya P, Rinschen MM, Khositseth S, Braucht DW, Chou CL, Pisitkun T, Nelson RD, Knepper MA. Systems-level analysis of cell-specific AQP2 gene expression in renal collecting duct. Proc Natl Acad Sci U S A. 2009 Feb 17;106(7):2441-6.

แหล่งทุน National Institutes of Health, Maryland, USA

31. Gonzales PA, Pisitkun T, Hoffert JD, Tchapyjnikov D, Star RA, Kleta R, Wang NS, Knepper MA. Large-scale proteomics and phosphoproteomics of urinary exosomes. J Am Soc Nephrol. 2009 Feb;20(2):363-79.

แหล่งทุน National Institutes of Health, Maryland, USA

32. Sachs AN, Pisitkun T, Hoffert JD, Yu MJ, Knepper MA. LC-MS/MS analysis of differential centrifugation fractions from native inner medullary collecting duct of rat. Am J Physiol Renal Physiol. 2008 Dec;295(6):F1799-806.

แหล่งทุน National Institutes of Health, Maryland, USA

33. Hoffert JD, Fenton RA, Moeller HB, Simons B, Tchapyjnikov D, McDill BW, Yu MJ, Pisitkun T, Chen F, Knepper MA. Vasopressin-stimulated increase in phosphorylation at ser-269 potentiates plasma membrane retention of aquaporin-2. J Biol Chem. 2008 Sep 5;283(36):24617-27.

แหล่งทุน National Institutes of Health, Maryland, USA

34. Pisitkun T, Jacob V, Schleicher SM, Chou CL, Yu MJ, Knepper MA. Akt and ERK1/2 pathways are components of the vasopressin signaling network in rat native IMCD. Am J Physiol Renal Physiol. 2008 Oct;295(4):F1030-43.

แหล่งทุน National Institutes of Health, Maryland, USA

35. Yu MJ, Pisitkun T, Wang G, Aranda JF, Gonzales PA, Tchapyjnikov D, Shen RF, Alonso MA, Knepper MA. Large-scale quantitative LC-MS/MS analysis of detergent-resistant membrane proteins from rat renal collecting duct. Am J Physiol Cell Physiol. 2008 Sep;295(3):C661-78.

แหล่งทุน National Institutes of Health, Maryland, USA

36. Ruttenberg BE, Pisitkun T, Knepper MA, Hoffert JD. PhosphoScore: an open-source phosphorylation site assignment tool for MSn data. J Proteome Res. 2008 Jul;7(7):3054-9.

แหล่งทุน National Institutes of Health, Maryland, USA

37. Hao JJ, Wang G, Pisitkun T, Patino-Lopez G, Knepper MA, Shen RF, Shaw S. Enrichment of distinct microfilament-associated and GTP-binding-proteins in membrane/microvilli fractions from lymphoid cells. J Proteome Res. 2008 Jul 3;7(7):2911-2927.

แหล่งทุน National Institutes of Health, Maryland, USA

38. Uawithya P, Pisitkun T, Ruttenberg BE, Knepper MA. Transcriptional profiling of native inner medullary collecting duct cells from rat kidney. Physiol Genomics. 2008 Jan 17;32(2):229-253.

แหล่งทุน National Institutes of Health, Maryland, USA

39. Hoffert JD, Wang G, Pisitkun T, Shen RF, Knepper MA. An automated platform for analysis of phosphoproteomic datasets: application to kidney collecting duct phosphoproteins. J Proteome Res. 2007 Sep;6(9):3501-8.

แหล่งทุน National Institutes of Health, Maryland, USA

40. Dvorak MM, De Joussineau C, Carter DH, Pisitkun T, Knepper MA, Gamba G, Kemp PJ, Riccardi D. Thiazide diuretics directly induce osteoblast differentiation and mineralized nodule formation by interacting with a sodium chloride co-transporter in bone. J Am Soc Nephrol. 2007 Sep;18(9):2509-16.

แหล่งทุน National Institutes of Health, Maryland, USA

41. Cheruvanky A, Zhou H, Pisitkun T, Kopp JB, Knepper MA, Yuen PS, Star RA. Rapid isolation of urinary exosomal biomarkers using a nanomembrane ultrafiltration concentrator. Am J Physiol Renal Physiol. 2007 May;292(5):F1657-61.

แหล่งทุน National Institutes of Health, Maryland, USA

42. Hoffert JD, Nielsen J, Yu MJ, Pisitkun T, Schleicher SM, Nielsen S, Knepper MA. Dynamics of aquaporin-2 serine-261 phosphorylation in response to short-term vasopressin treatment in collecting duct. Am J Physiol Renal Physiol. 2007 Feb;292(2):F691-700.

แหล่งทุน National Institutes of Health, Maryland, USA

43. Zhou H, Pisitkun T, Aponte A, Yuen PS, Hoffert JD, Yasuda H, Hu X, Chawla L, Shen RF, Knepper MA, Star RA. Exosomal Fetuin-A identified by proteomics: a novel urinary biomarker for detecting acute kidney injury. Kidney Int. 2006 Nov;70(10):1847-57.

แหล่งทุน National Institutes of Health, Maryland, USA

44. Wang G, Wu WW, Pisitkun T, Hoffert JD, Knepper MA, Shen RF. Automated quantification tool for high-throughput proteomics using stable isotope labeling and LC-MSn. Anal Chem. 2006 Aug 15;78(16):5752-61.

แหล่งทุน National Institutes of Health, Maryland, USA

45. Yu MJ, Pisitkun T, Wang G, Shen RF, Knepper MA. LC-MS/MS analysis of apical and basolateral plasma membranes of rat renal collecting duct cells. Mol Cell Proteomics. 2006 Nov;5(11):2131-2145.

แหล่งทุน National Institutes of Health, Maryland, USA

46. Hoffert JD, Pisitkun T, Wang G, Shen RF, Knepper MA. Quantitative phosphoproteomics of vasopressin-sensitive renal cells: regulation of aquaporin-2 phosphorylation at two sites. Proc Natl Acad Sci U S A. 2006 May 2;103(18):7159-64.

แหล่งทุน National Institutes of Health, Maryland, USA

47. Zhou H, Yuen PS, Pisitkun T, Gonzales PA, Yasuda H, Dear JW, Gross P, Knepper MA, Star RA. Collection, storage, preservation, and normalization of human urinary exosomes for biomarker discovery. Kidney Int. 2006 Apr;69(8):1471-6.

แหล่งทุน National Institutes of Health, Maryland, USA

48. Pisitkun T, Bieniek J, Tchapyjnikov D, Wang G, Wu WW, Shen RF, Knepper MA. High-throughput identification of IMCD proteins using LC-MS/MS. Physiol Genomics. 2006 Apr 13;25(2):263-76.

แหล่งทุน National Institutes of Health, Maryland, USA

49. Ricci Z, Salvatori G, Bonello M, Pisitkun T, Bolgan I, D'Amico G, Dan M, Piccinni P, Ronco C. In vivo validation of the adequacy calculator for continuous renal replacement therapies. Critical Care. 2005;9:R266-R273.

แหล่งทุน Faculty of Medicine, Chulalongkorn University

50. Barile M, Pisitkun T, Yu MJ, Chou CL, Verbalis MJ, Shen RF, Knepper MA. Large-scale protein identification in intracellular aquaporin-2 vesicles from renal inner medullary collecting duct. Mol Cell Proteomics. 2005 Aug;4(8):1095-1106.

แหล่งทุน National Institutes of Health, Maryland, USA

51. Praditpornsilpa K, Buranasot S, Bhokaisuwan N, Avihingsanon Y, Pisitkun T, Kansanabuch T, Eiam-Ong S, Chusil S, Intarakumtornchai T, Tungsanga K. Recovery from anti-recombinant-human-erythropoietin associated pure red cell aplasia in end-stage renal disease patients after renal transplantation. Nephrol Dial Transplant. 2005 Mar;20(3):626-30.

แหล่งทุน Faculty of Medicine, Chulalongkorn University

52. Punakabutra N, Nunthapisud P, Pisitkun T, Tiranathanagul K, Tungsanga K, Eiam-Ong S. Comparison of different culture methods on bacterial recovery in hemodialysis fluids. J Med Assoc Thai. 2004 Nov;87(11):1361-7.

แหล่งทุน Faculty of Medicine, Chulalongkorn University

53. Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. Proc Natl Acad Sci U S A. 2004 Sep 7;101(36):13368-73.

แหล่งทุน National Institutes of Health, Maryland, USA

54. Praditpornsilpa K, Avihingsanon Y, Kupatawintu P, Songpanich S, Pisitkun T, Kansanabuch T, Eiam-Ong S, Chusil S, O-Charoen R, Tungsanga K. Monitoring of T-cell subsets in patients treated with anti-CD 25 antibody. Transplant Proc. 2004 Mar;36(2 Suppl):487S-491S.

แหล่งทุน Faculty of Medicine, Chulalongkorn University

55. Pisitkun T, Tiranathanagul K, Poulin S, Bonello M, Salvatori G, D'Intini V, Ricci Z, Bellomo R, Ronco C. A practical tool for determining the adequacy of renal replacement therapy in acute renal failure patients. Contrib Nephrol. 2004;144:329-49.

แหล่งทุน Faculty of Medicine, Chulalongkorn University

56. Pisitkun T, Eiam-Ong S, Tiranathanagul K, Sakunsrijinda C, Manotham K, Hanvivatvong O, Suntaranuson P, Praditpornsilpa K, Chusil S, Tungsanga K. Convective-controlled double high flux hemodiafiltration: a novel blood purification modality. Int J Artif Organs. 2004 Mar;27(3):195-204.

แหล่งทุน Faculty of Medicine, Chulalongkorn University

57. Pisitkun T, Eiam-Ong S, Chusil S, Praditpornsilpa K, Pansin P, Tungsanga K. The role of C4 and AUC0-4 in monitoring of tacrolimus in stable kidney transplant patients. Transplant Proc 2002;34:3173-5.

แหล่งทุน Faculty of Medicine, Chulalongkorn University

58. Booranalertpaisarn V, Pisitkun T, Hlorsuwanakul R, Eiam-Ong S. Clinical trials of cyclosporine, FK 506 (tacrolimus), and sirolimus in kidney transplantation patients. J Nephrol Soc Thai. 2001;7(3):320-37.

แหล่งทุน Faculty of Medicine, Chulalongkorn University

59. Radin MJ, Yu MJ, Stoedkilde L, Miller RL, Hoffert JD, Frokiaer J, Pisitkun T, Knepper MA. Aquaporin-2 regulation in health and disease. Vet Clin Pathol. 2012 Dec;41(4):455-70. doi: 10.1111/j.1939-165x.2012.00488.x. Epub 2012 Nov 6.

แหล่งทุน National Institutes of Health, Maryland, USA

60. Huling JC, Pisitkun T, Song JH, Yu MJ, Hoffert JD, Knepper MA. Gene Expression Databases for Kidney Epithelial Cells. Am J Physiol Renal Physiol. 2012 Feb;302(4):F401-7.

แหล่งทุน National Institutes of Health, Maryland, USA

61. van Balkom BW, Pisitkun T, Verhaar MC, Knepper MA. Exosomes and the kidney: prospects for diagnosis and therapy of renal diseases. Kidney Int. 2011 Dec;80(11):1138-45.

แหล่งทุน National Institutes of Health, Maryland, USA

62. Hoffert JD, Pisitkun T, Knepper MA. Phosphoproteomics of vasopressin signaling in the kidney. Expert Rev Proteomics. 2011 Apr;8(2):157-63.

แหล่งทุน National Institutes of Health, Maryland, USA

63. Gonzales PA, Zhou H, Pisitkun T, Wang NS, Star RA, Knepper MA, Yuen PS. Isolation and purification of exosomes in urine. Methods Mol Biol. 2010;641:89-99.

แหล่งทุน National Institutes of Health, Maryland, USA

64. Hoorn EJ, Pisitkun T, Yu MJ, Knepper MA. Proteomic approaches for the study of cell signaling in the renal collecting duct. Contrib Nephrol. 2008;160:172-85.

แหล่งทุน National Institutes of Health, Maryland, USA

65. Gonzales P, Pisitkun T, Knepper MA. Urinary exosomes: is there a future? Nephrol Dial Transplant. 2008 Jun;23(6):1799-801.

แหล่งทุน National Institutes of Health, Maryland, USA

66. Pisitkun T, Hoffert JD, Yu MJ, Knepper MA. Tandem mass spectrometry in physiology. Physiology (Bethesda). 2007 Dec;22:390-400.

แหล่งทุน National Institutes of Health, Maryland, USA

67. Knepper MA, Pisitkun T. Exosomes in urine: Who would have thought...? Kidney Int. 2007 Nov;72(9):1043-5.

แหล่งทุน National Institutes of Health, Maryland, USA

68. Pisitkun T, Johnstone R, Knepper MA. Discovery of urinary biomarkers. Mol Cell Proteomics. 2006 Oct;5(10):1760-1771.

แหล่งทุน National Institutes of Health, Maryland, USA

69. Hoorn EJ, Pisitkun T, Zietse R, Gross P, Frokiaer J, Wang NS, Gonzales PA, Star RA, Knepper MA. Prospects for urinary proteomics: exosomes as a source of urinary biomarkers. Nephrology (Carlton). 2005 Jun;10(3):283-90.

แหล่งทุน National Institutes of Health, Maryland, USA

70. Ronco C, Inguaggiato P, D'Intini V, Cole L, Bellomo R, Poulin S, Bordoni V, Crepaldi C, Gastaldon F, Brendolan A, Pisitkun T, Tiranathanagul K. The role of extracorporeal therapies in sepsis. J Nephrol. 2003 Nov-Dec;16 Suppl 7:S34-41.

แหล่งทุน Faculty of Medicine, Chulalongkorn University

7.3 งานวิจัยที่กำลังทำ : ชื่อข้อเสนอการวิจัย แหล่งทุน และสถานภาพในการทำวิจัยว่าได้ทำการวิจัยลุล่วงแล้วประมาณร้อยละเท่าใด

ชื่อข้อเสนอการวิจัย Systems biology of aberrant targeting and polarization of membrane proteins in disease

แหล่งทุน Lundbeck Foundation (2012): total award 500,000 Danish krone

การวิจัยลุล่วงแล้วประมาณร้อยละ 25

2.ผู้ร่วมโครงการวิจัย

1. ชื่อ - นามสกุล (ภาษาไทย) ประภาพร พิสิษฐ์กุล

   ชื่อ - นามสกุล (ภาษาอังกฤษ) Prapaporn Pisitkun

2. เลขหมายบัตรประจำตัวประชาชน 3100100484089

   ตำแหน่งปัจจุบัน อาจารย์

   เวลาที่ใช้ทำวิจัย 40 (ชั่วโมง : สัปดาห์)

3. ตำแหน่ง อาจารย์

4. ภาควิชาภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล

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5. ประวัติการศึกษา

2002 Board of Clinical Rheumatology, Thailand

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1995 M.D., Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

1993 Bachelor of Science, Mahidol University, Bangkok, Thailand

6. สาขาวิชาการที่มีความชำนาญพิเศษ (แตกต่างจากวุฒิการศึกษา) ระบุสาขาวิชาการ

อิมมูโนวิทยา โรคออโตอิมมูน การวิจัยในสัตว์ทดลอง

7. ประสบการณ์ที่เกี่ยวข้องกับการบริหารงานวิจัยทั้งภายในและภายนอกประเทศ โดยระบุสถานภาพในการทำการวิจัยว่าเป็นผู้อำนายการแผนงานวิจัย หัวหน้าโครงการวิจัย หรือผู้ร่วมวิจัยในแต่ละข้อเสนอการวิจัย เป็นต้น

7.1 ผู้อำนวยการแผนงานวิจัย : -

7.2 หัวหน้าโครงการวิจัย: -

7.3 ผู้ร่วมงานวิจัย: -

7.4 โครงการที่ทำเสร็จแล้ว :

1. Benjachat T, Tongyoo P, Tantivitayakul P, Somparn P, Hirankarn N, Prom-On S, Pisitkun P, Leelahavanichkul A, Avihingsanon Y*, Townamchai N. Biomarkers for Refractory Lupus Nephritis: A microarrays study of kidney tissue. Int. J. Mol. Sci. 2015 (in press).

2. Sun C*, Schattgen SA*, Pisitkun P*, Jorgensen JP*, Hilterbrand AT, Wang LJ, West JA, Hansen K, Horan KA, Jakobsen MR, O'Hare P, Adler H, Sun R, Ploegh HL, Damania B, Upton JW, Fitzgerald KA, Paludan SR. Evasion of innate cytosolic DNA sensing by a gammaherpesvirus facilitates establishment of latent infection. J Immunol. 2015 Feb 15;194(4):1819-31.

3. Ha HL, Wang H, Pisitkun P, Kim JC, Tassi I, Tang W, Morasso MI, Udey MC, Siebenlist U. IL-17 drives psoriatic inflammation via distinct, target cell-specific mechanisms. Proc Natl Acad Sci U S A. 2014 Aug 19;111(33):E3422-31.

4. Pisitkun P, Ha HL, Wang H, Claudio E, Tivy CC, Zhou H, Mayadas TN, Illei GG, Siebenlist U. IL-17 cytokines are critical in development of fatal lupus glomerulonephritis. Immunity. 2012 Oct 30. [Epub ahead of print]

5. Pisitkun P*, Walsh ER*, Voynova E, Deane JA, Scott BL, Caspi RR, Bolland S. Dual signaling by innate and adaptive immune receptors is required for Tlr7-induced B cell-mediated autoimmunity. Proc Natl Acad Sci U S A. 2012 Oct 2; 109(40): 16276-81.

6. Pisitkun P, Claudio E, Ren N, Wang H, Siebenlist U. The adaptor protein CIKS/Act1 is necessary for collagen-induced arthritis, and it contributes to the production of collagen-specific antibody. Arthritis Rheum. 2010 Nov; 62(11):3334-44.

7. Richter K, Brar Sukdev, Ray M, Pisitkun P, Bolland S, Verkoczy L, Diaz M. Speckled-like pattern in the germinal center (SLIP-GC): A nuclear GTPase highly expressed in AID-positive lymphomas and in germinal center B cells. J Biol Chem. 2009 Oct; 284(44):30652-61.

8. Deane JA, Pisitkun P, Barrett RS, Feigenbaum L, Town Terrence, Ward JM, Flavell RA, Bolland S. Control of Toll-like Receptor 7 Expression Is Essential to Restrict Autoimmunity and Dendritic cell Proliferation. Immunity. 2007 Nov; 27(5):801-10.

9. Parkpian V, Verasertniyom O, Vanichapuntu M, Totemchokchyakarn K, Nantiruj K, Pisitkul P, et al. Specificity and sensitivity of anti-beta(2)-glycoprotein I as compared with anticardiolipin antibody and lupus anticoagulant in Thai systemic lupus erythematosus patients with clinical features of antiphospholipid syndrome. Clin Rheumatol. 2007 Oct; 26(10):1663-70.

10. Pisitkun P, Deane JA, Difilippantonio MJ, Tarasenko T, Satterthwaite AB, Bolland S. Autoreactive B Cell Responses to RNA-Related Antigens Due to TLR7 Gene Duplication. Science. 2006 Jun; 312(5780):1669-72.

11. Pakunpanya K, Verasertniyom O, Vanichapuntu M, Pisitkun P, et al. Incidence and clinical correlation of anticentromere antibody in Thai Patients. Clin Rheumatol. 2006 May; 25(3):325-8.

12. Pisitkun P, Pattarowas C, Siriwongpairat P, Totemchokchyakarn K, Nantiruj K, and Janwityanujit S. Reappraisal of cervical spine subluxation in Thai patients with rheumatoid arthritis. Clin Rheumatol. 2004 Feb; 23(1):14-8.

โครงการวิจัยย่อยที่ 2

"รายงานการวิจัย"เรื่อง "หนูทดลองโมเดลโรคเอสแอลอีโดยการขาดตัวรับ FcGRIIb เกิดความรุนแรงของการติดเชื้อในกระแสเลือดด้วยโมเดลการผูกและเจาะลำไส้ในหนูทดลองสูงขึ้นเนื่องจากภาวะ Macrophage exhaustion"

"Fc gamma receptor IIb deficient mice, a Systemic Lupus Erythematosus mouse model, susceptible to cecal ligation and puncture sepsis after preconditioned with double separated doses of endotoxin due to the prominent macrophage exhaustion"

บทคัดย่อภาษาไทย

การติดเชื้อแทรกซ้อนและซ้ำซ้อนพบได้บ่อยในผู้ป่วย เอสแอลอี ซึ่งเป็นสาเหตุการตายที่สำคัญในผู้ป่วย ผ้วิจัยเลียนแบบภาวะการติดเชื้อซ้ำซ้อนโดยการฉีด endotoxin 2 ครั้ง ห่างกัน 5 วัน แล้วจึงทำการผ่าตัดเจาะและผูกลำไส้ใหญ่เพื่อการะตุ้นการติดเชื้อในกระแสเลือดที่ 24 ชั่วโมงถัดไป ในหนูทดลองที่ขาดตัวรับชนิด FcGRIIb ซึ่งเป็นโมเดลของโรคเอสแอลอีโมเดลหนึ่ง พบว่าหนูทดลองกลุ่มเอสแอลอีเมื่อเทียบกับหนู wild type นั้น มีการตอบสนองด้วยการสร้างไซโตคายด์หลังการฉีด endotoxin ครั้งแรกสูงมาก แต่ต่ำมากหลังการฉีดครั้งที่ 2 เข้าได้กับ การเกิด immune-exhaustion ซึ่งพบว่าการกระตุ้นให้เกิดการติดเชื้อในกระแสเลือดหลังจากกระตุ้นด้วย endotoxin 2 ครั้งนั้น ส่งผลให้เกิดการติดเชื้อที่รุนแรงมากในหนูเอสแอลอี (พิจารณาจากค่าที่บ่งบอกการทำงานของตับและไตจากการเจาะเลือด จากระดับไซโตคายด์ จากจำนวนแบคทีเรียที่พบในอวัยวะต่างๆและอัตราการตาย) เนื่องจากแมคโครเฟส เป็นเซลที่มีความสำคัญอย่างยิ่งในการต่อสู้กับการติดเชื้อ จึงได้ทำการทดลองในเซลดังกล่าวพบว่า แมคโครเฟสของหนูเองแอลอีเมื่อเปรียบเทียบกับหนูปกติตอบสนองต่อ endotoxin ในครั้งแรกโดยการสร้างไซโตคายด์ในระดับที่สูงมาก แต่มีระดับต่ำมากคล้ายกับที่พบในสัตว์ทดลองหลังจากการกระตุ้นครั้งที่สอง เข้าได้กับภาวะ macrophage paralysis

ดังนั้น immune-exhaustion ในสัตว์ทดลองน่าจะเกิดจาก macrophage paralysis ผลการทดลองแสดงให้เห็นความสำคัญของการติดเชื้อซ้ำซ้อนในผู้ป่วยเอสแอลอี โดยเฉพาะอย่างยิ่งในผู้ที่มีความผิปกติของยีน FcGRIIb การค้นหาความผิดปกติของ FcGRIIb ในผู้ป่วยโรคเอสแอลอีอาจจะมีบทบาทในการเฝ้าระวังและป้องกันการติดเชื้อในกระแสเลือดในผู้ป่วยเองแอลอีอันเป็นสาเหตุการตายที่สำคัญของโรค

Abstract

Repeated bacterial infection in patients with Systemic Lupus Erythematosus (SLE) is common and sepsis is the leading causes of death. Despite proper responses to a single bacterial infection, the repeated infection might lead to immune exhaustion and severe sepsis. Then the bacterial susceptibility was tested with cecal ligation and puncture (CLP) after immune exhaustion induced by the 2-separated-doses of endotoxin (LPS) in FcGRIIb-/- mice and wild type (WT) control.

In the comparison with wild type group, the prominent serum cytokine after 1st LPS injection followed by the apparently lower cytokines after 2nd LPS administration, cytokine exhaustion, was demonstrated in FcGRIIb-/- mice. Subsequently, CLP was conducted after double doses of LPS preconditioning to test the immune suppression. Indeed, a higher mortality rate and a more severe sepsis (bacterial burdens, serum cytokines and organs injury) at 18h of CLP demonstrated in FcGRIIb-/- mice. Because macrophages are the major immune cells responsible for sepsis immune responses, we tested *in vitro*. Interestingly, the stimulation with separated 2 doses of LPS in bone marrow-derived macrophage from FcGRIIb-/- mice showed the higher cytokines responses after the 1st LPS stimulation in comparison with WT cells but the cytokines level were lower than WT cells after the 2nd LPS stimulation, supplementary to the *in vivo* results.

In conclusion, macrophage exhaustion was easier inducible in FcGRIIb-/- cells in parallel to the immune paralysis, highly susceptible to CLP, in FcGRIIb-/- mice compare with wild type group. These implied the importance of the repeated infections in patients with SLE, especially with FcGRIIb polymorphisms.

6. คำอธิบายสัญญาลักษณ์และคำย่อที่ใช้ในการวิจัย (List of Abbreviations)

SLE, Systemic lupus Erythematosus; FcGRIIb, Fc gamma receptor IIb; LPS, endotoxin; CLP, cecalligation and puncture model; UPCI, urine protein creatinine index; LDH, lactate dehydrogenase

ส่วนประกอบเนื้อเรื่อง

1. บทนำ (Introduction) ซึ่งกล่าวถึงเนื้อหาของเรื่องที่เคยมีผู้ทำการวิจัยมาก่อน ความสำคัญและที่มาของปัญหา วัตถุประสงค์และขอบเขตการวิจัย วิธีดำเนินการวิจัยโดยสรุปทฤษฎีและ/หรือแนวทางความคิดที่นำมาใช้ในการวิจัย ประโยชน์ที่คาดว่าจะได้รับ ฯลฯ

Systemic Lupus Erythematosus (SLE), the autoimmune disease with multi-factorial pathogenesis (1, 2) leads to multi-organs injury, showed a higher prevalence in Asia in comparison with other regions of the world (3-5). The defect of Fc gamma receptor IIb (FcGRIIb), the only inhibitory signaling receptors in the FcGR family, is one of the genetic susceptibility to SLE (2, 6). Interestingly, FcGRIIb polymorphisms also demonstrated the high prevalence in Asia which might due to the protective effect of the gene for malarial infection (7). Coincidentally, the association with FcGRIIb polymorphisms in patients with SLE in Asia Pacific region is also common (3-5, 8). Perhaps FcGRIIb polymorphisms could protect malaria in this region but, on other side of the coin, people with this immunological defect might easier develop SLE. In any case, sepsis, the systemic immune responses to the severe infection, is one of the important causes of death in patients with SLE (9). Indeed, the high susceptibility to bacterial sepsis in patients with SLE is well-known (9-13). However, there are debates whether the susceptibility to infection in patients with SLE is due to the *de novo* defects of immune response or immunosuppressive drugs. Unfortunately, the data on untreated symptomatic patients with SLE is very limited (14). Hence, the studies of infection in FcGRIIb-/- mice, one of the established SLE mouse model (6), could be resemble to untreated patients with SLE, especially with FcGRIIb polymorphisms.

FcGRs binds with Fc portion of immunoglobulin mediate antigen uptake and cellular responses (15). In the mouse, FcGRs are classified into three activation receptors (FcGRI, FcGRIII, FcGRIV) and only one inhibitory receptor (FcGRIIb) (16). The deficiency of all classes of FcGR in mice (FcGR -/-) protected from sepsis (17) and FcGRIIb-/- mice response well to the gram positive bacterial infection due to the effective bacterial killing (18). Nevertheless, a more severe sepsis with the cytokines storm demonstrated in FcGRIIb-/- mice with the bacterial antigen preconditioning before bacterial administration. This data supported the overshoot cytokines responses after the repeated antigen exposures due to the lack of inhibitory signaling in these mice (18). On the other hand, FcGRIIb-/- mice are protected from Plasmodium and Mycobacterium infection (7, 19), the

inhibitory signaling defect seems to enhance the activating signaling and show a benefit in these infections. However, the susceptibility of FcGRIIb-/- mice to polymicrobial sepsis is never tested.

Interestingly, sepsis-induced immune exhaustion or immunoparalysis, the high susceptibility to secondary infection after sepsis, has been recognized as an important sepsis complication (20, 21) and was demonstrated by several mouse and human models (22-26). In contrast, the preconditioning of LPS, a single or multiple doses, for 24h before CLP ameliorates sepsis severity in wild type mice has been showed in previous publications (27, 28). Despite the demonstrated protective effect to sepsis after 1 day of LPS preconditioning, we hypothesized that the immune exhaustion after LPS administration might existed in the earlier period and the repeated endotoxin exposure might mimic the repeated infection in patients. Subsequently, we selected the preconditioning with double doses of LPS with 5 days separation followed by CLP at 12h after the 2^{nd} dose of LPS to demonstrate the immune exhaustion in our models. Of note, half-life of the important LPS-induced cytokines (TNF-α, IL-6 and IL-10) is approximately 0.5-1.5h (29), then at 12h, approximately 8 times of the half-life, should be adequate for avoiding the effect of these cytokines to the subsequent CLP surgery.

On the other hand, the lower macrophage immune responses, especially cytokines production, after repeated LPS activation is demonstrated, and was known by several terms such as “macrophage paralysis” or “macrophage exhaustion” or “macrophage tolerance” or “endotoxin tolerance” (30-32). Although, the association between immunoparalysis and macrophage exhaustion is not clearly demonstrated (31), it is possible that macrophage exhaustion might cause ineffective organisms clearance and increase infection susceptibility compatible with the definition of “immunoparalysis”.

Indeed, macrophage contains both activating and inhibitory FcGRs which competing for immune complex ligands and the direction of this balance determines the direction of the cell responses (16, 33). We hypothesize that the defect in the inhibitory signaling of FcGRIIb-/- mice might result in a prominent response but is easier exhausted after repeated stimulation. Then we test immunoparalysis of FcGRIIb-/- mice *in vivo* and macrophage exhaustion *in vitro*, respectively.

2. เนื้อเรื่อง (Main Body) ซึ่งกล่าวถึงรายละเอียดเกี่ยวกับวิธีดำเนินการวิจัย (Materials & Method) ผลการวิจัย (Results) ฯลฯ

Materials and Methods

Animal and animal models

FcGRIIb-/- mice on C57BL/6 background were provided by Bolland S. (NIH, Maryland, USA). Other mice were purchased from the National Laboratory Animal Center, Nakornpathom, Thailand. Female, 8- and 24-week-old C57BL/6 mice were used in the experiments. The animal protocols were approved by Faculty of Medicine, Chulalongkorn University followed the National Institutes of Health (NIH) criteria.

Cecal ligation and puncture model

Polymicrobial sepsis was induced by Cecal ligation and puncture model (CLP) slightly modified from the previous publication (29). Briefly, cecum were ligated at 10 mm from cecal tip with silk 2-0, punctured twice with a 21-gauge needle then gently squeezed to expel a small amount of fecal materials through an abdominal incision under isoflurane anesthesia. The incisions were closed with 2 layers by nylon 4-0 and normal saline (NSS) at 2 ml/kg was administered subcutaneously for the fluid replacement.

Cecal ligation and puncture with endotoxin pre-conditioning model

Because LPS induced-immuno-suppression is demonstrated (34, 35) and used as a sepsis-induced immunoparalysis model (26), we follow the principle in our experiments. In our model, the immunoparalysis, a condition susceptible to an infection, was tested by the severity of polymicrobial infection from CLP surgery. Endotoxin (LPS) of *Escherichia coli* 026:B6 (Sigma-Aldrich, St. Louis, USA) was administered intraperitoneally at 5 days (-120h) and 12h (-12h) before CLP surgery at the dose of 0.8 g/kg (approximately 20 μg per 25 g mouse) and 4 g/kg (approximately 100 μg per 25 g mouse), respectively. Subsequently, CLP was performed as previously mentioned.

To measure inflammatory cytokines after LPS injection, 50 μl of blood was collected through tail vein nicking at 0h (2h before LPS administration) and at 1, 3 and 6h after. In addition, in separated experiments, blood from tail vein nicking was also collected before CLP (0h) and at 3h and 6h to measure time-courses of bacterial burdens and serum cytokines after CLP surgery. Otherwise, blood was collected through cardiac puncture at sacrifice time under isoflurane anesthesia at 18h or 96h after CLP for sepsis injury analysis or survival test, respectively.

Blood chemistry, supernatant media analysis and urine protein

For the natural history of FcGRIIb-/- mice, serum from tail vein nicking and spot urine was collected once a month from 2 to 12-month-old. Serum and urine creatinine were measured by (QuantiChrom Creatinine Assay, DICT-500, BioAssay, CA, USA). Spot urine protein was measured by Bradford protein assay. Urine protein creatinine index (UPCI), a representative of 24h urine protein, were measured from spot urine by equation; urine protein/urine creatinine.

Serum cytokines after LPS were measured by Luminex-based multiplex technology multi-analysis panels 8-plex cytokines assay (Bioplex, Bio-RAD, CA, USA) to explore the panel of pro and anti -inflammatory cytokines (TNF-α, IL-6, IL-1β, IFN-γ and IL-2, IL-4, IL-5, IL-10, respectively) according to the manufacturer's protocol. Then the selected important cytokines (TNF-α, IL-6, IL-10) were measured by ELISA assay (ReproTech, NJ, USA) in supernatant media and in mouse serum after CLP surgery. Organs injury was determined by blood urea nitrogen (QuantiChrom Urea Assay, DIUR-500, BioAssay), serum creatinine (Scr) (QuantiChrom Creatinine Assay, DICT-500, BiaAssay), alanine transaminase (ALT) (EnzyChrom ALT assay, EALT-100, BioAssay) and lactate dehydrogenase (LDH) (EnzyChrom LDH assay, EDLC-100, BioAssay). Blood bacterial burdens were determined by plating a serial volume of blood into blood agar (Oxoid, Hampshire, UK) at 37°C then counted bacterial colonies after 24h of incubation. For blood polymorphonuclear cell (PMN) and mononuclear cell count, 5 μl of blood mixed in 85 μl of 3% acetic acid for the hemolytic reaction and total leukocytes was counted by a hemocytometer. In parallel, blood smeared on a glass slide was stained by Wright stain and counted with x100 magnification in 100 fields to determine the percentage of PMN and mononuclear cells. The total number of cells was calculated by total leukocyte count from hemocytometer multiplied by the percentage of cells from the Wright stain glass slide.

Anti-dsDNA antibodies

Anti-dsDNA antibodies were measured by coating ELISA plates with salmon sperm DNA as published previously (36). In short, salmon sperm DNA (Life Technologies, Invitrogen™, MA, USA) passage through a 45-mm filter (Minisart, Sartorius, Germany) for selecting double stranded DNA then coated into ELISA plate with the dose at 100 μg/plate. The plates were dry, blocked and incubated with serial dilutions of serum for 1 hour at 37°C then peroxidase conjugated Fab'2 goat

anti-mouse IgG 1/2,000 in 1% bovine serum albumin (BSA) in phosphate buffer solution (PBS) followed by TMB peroxidase substrate (Biolegend, California, USA). The plate was developed in the dark room for 10 min then added TMB stop solution and read with microplate photometers with a wavelength at 410 nm.

Bone Marrow-Derived Macrophages

Macrophages were derived from bone marrows (BM) follow the established procedure (37). In short, BM cells from FcGRIIb-/- and wild type mice obtained from femurs were centrifuged at 1,000 rpm in 4 °C for 10 min. Then cell were incubated in high glucose DMEM supplement with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, HEPES with sodium pyruvate and 20% L929-conditioned media in a humidified 5% CO_2 incubator at 37 °C for 7 days. The cells were harvested at the end of the culture period using very cold PBS and confirmed macrophage phenotype with anti-F4/80 and anti-CD11c antibodies (BioLegend, CA, USA).

Macrophage endotoxin tolerance protocol

Macrophage endotoxin tolerance protocol followed the protocols from the previous publications (38, 39). Briefly, endotoxin (LPS) *Escherichia coli* 026:B6 (Sigma) at 10 or 100 ng/ml was used to activate macrophage $1x10^5$ cells/well in 96 well polystyrene tissue culture plate. To see the difference between single or double LPS stimulations, 2 groups of experiments were performed. For the single LPS stimulation (N/LPS), there was no endotoxin at the 1st 24h of the incubation then the plate was washed with phosphate buffer solution (PBS), refilled fresh media and treated with LPS at 10 ng/ml (N/LPS10) or 100 ng/ml (N/LPS100). For the double LPS stimulation (LPS/LPS), LPS at 10 or 100 ng/ml was treated for the 1st 24h and treated with the 2nd dose of LPS at 10 ng/ml or 100ng/ml as indicated. The culture supernatant was collected at 1, 2, 4, 6 and 24h after the 2nd LPS incubation in all groups and stored at -80 °C until cytokine determination by ELISA assays (ReproTech). After the incubation, cell viability was measured by MTS assay (One Solution Cell Proliferation Assay, Promega Corporation, WI, USA) according to the manufacturer's instruction (40). In short, 20 μl of MTS was added to the culture plates for 2h at 37°C in 5% CO2 incubator then read with microplate photometers with a wavelength at 450 nm. All *in vitro* experiments demonstrated cell viability more than 95% (data not showed).

Macrophage intracellular killing activity and phagocytosis protocol

The protocol followed the previous publication (41). BM derived macrophage at $1x10^5$ cells in 200 ul of DMEM per well were dispensed into the flat bottom 96-well plate and incubated at 37 °C in a humidified 5% (v/v) CO_2 incubator for 24 h, before gently washing with culture media to remove non-adherent cells. Subsequently, the cells with endotoxin (LPS) *Escherichia coli* 026:B6 (Sigma) at 10 or 1,000 ng/ml, LPS10 or LPS1000, respectively, and incubated with $1x\ 10^7$CFU of *E.Coli* per well.

Then after 15 min of incubation, supernatants were aspirated and cells were washed gently with DMEM to remove un-ingested microorganisms. The supernatant and well washing fluids, containing the non-phagocytized *E.Coli*, were combined, plated in serial dilutions on Tryptic soy agar plates and counted for bacterial colonies for the representative of the non-phagocytic bacteria which reversed correlated with the phagocytic activity. On the other hand, the cellular part, phagocytosed macrophage, was further incubated with 200 ul of DMEM for 2h to determine intracellular bacterial killing activity. The wells were gently scraped and washed with 200 ml distilled H_2O to induce cell lysis and the serial dilution of the lysate were plated on Tryptic soy agar, incubated at 37 °C for 16 h and determine the bacterial colony count. The number of bacteria from the cell lysate represented the intracellular killing activity.

Statistical analysis

Data are shown as the mean ± SE and differences between groups were examined for statistical significance using the unpaired Student t-test or one-way analysis of variance (ANOVA) with Tukey's comparison test for the analysis of experiments with 2 and 3 groups, respectively. Survival analyses were evaluated using the log-rank test by observation and recorded every 6–24h then all mice were sacrifice at 96h after CLP. P values < 0.05 were considered statistically significant. SPSS 11.5 software (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis.

Result

Fc gamma receptor IIb deficient mice susceptible to cecal ligation and puncture sepsis in symptomatic SLE mice and asymptomatic SLE group preconditioned with LPS

At 24-weeks-old, FcGRIIb-/- mice showed increased anti-dsDNA with proteinuria but normal kidney function as evaluated by serum creatinine (Scr) (Fig 1). This natural history allows the experiments in 2 groups of mice in correspondence with patients with SLE; asymptomatic genetic prone group (8-weeks-old) and symptomatic proteinuria group (24-weeks-old). To see the susceptibility to bacterial sepsis without LPS preconditioning in these 2 groups, CLP surgery was performed in comparison with age-matched wild type control. In the absence of LPS, only symptomatic, but not asymptomatic, FcGRIIb-/- mice showed higher sepsis mortality rate compare with wild type (Fig 2A, B) supports the correlation between SLE disease activity and infection susceptibility (9).

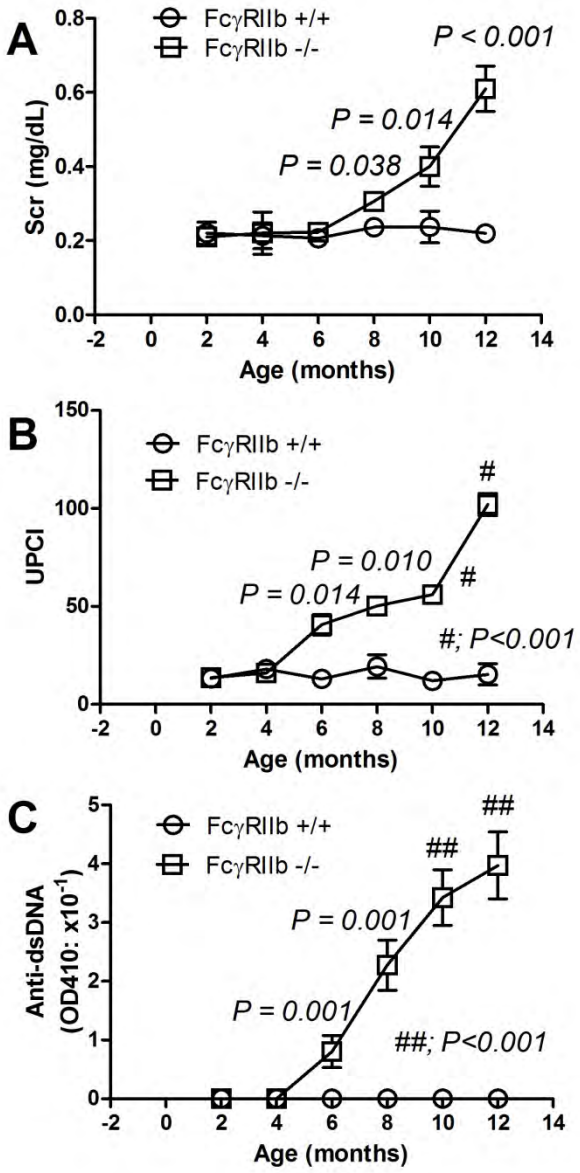

Figure 1. The natural history of FcGRIIb-/- and wild type (FcGRIIb+/+) mice as determined by serum creatinine (Scr), urine protein creatinine index (UPCI) and anti-dsDNA (n=4-5/groups).

Then we test the effect of LPS preconditioning in asymptomatic mice and determine the severity of immunoparalysis by the mortality rate of CLP surgery (20, 21).

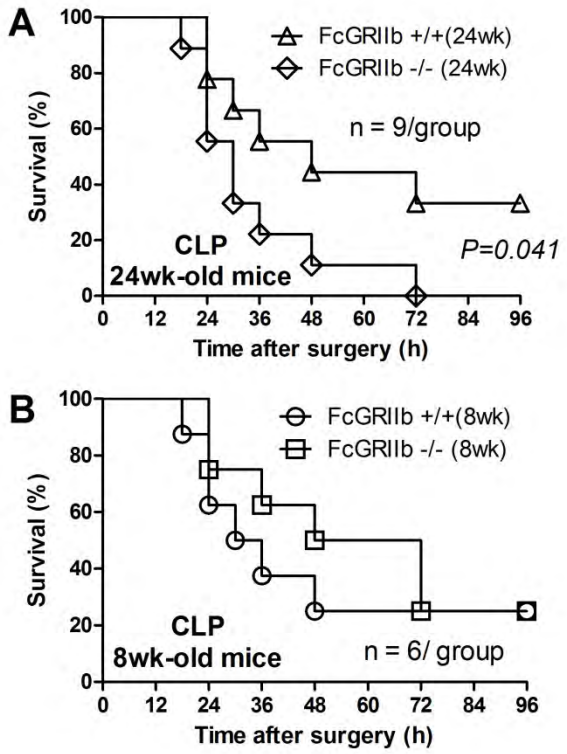

Figure 2. *Survival analysis of cecal ligation and puncture (CLP) sepsis surgery in asymptomatic FcGRIIb-/- mice (8-wk-old) (A) and symptomatic, proteinuria positive but normal serum creatinine, FcGRIIb-/- mice (24-wk-old) (B) in comparison with age-matched wild type mice (FcGRIIb+/+).*

Although the high CLP mortality rate found in both wild type and FcGRIIb-/- mice after LPS-preconditioning, FcGRIIb-/- showed the higher mortality rate. Whereas all wild type and FcGRIIb-/- mice die within 72h and 36h, respectively, in CLP with LPS, the survival rate at 30% and 22% found in wild type and FcGRIIb-/- mice, respectively, in CLP alone (Fig 3A, B). These results supported the immunoparalysis occur in both wild type and FcGRIIb-/- mice after LPS preconditioning but FcGRIIb-/- mice showed the more severe immunoparalysis (Fig 3C).

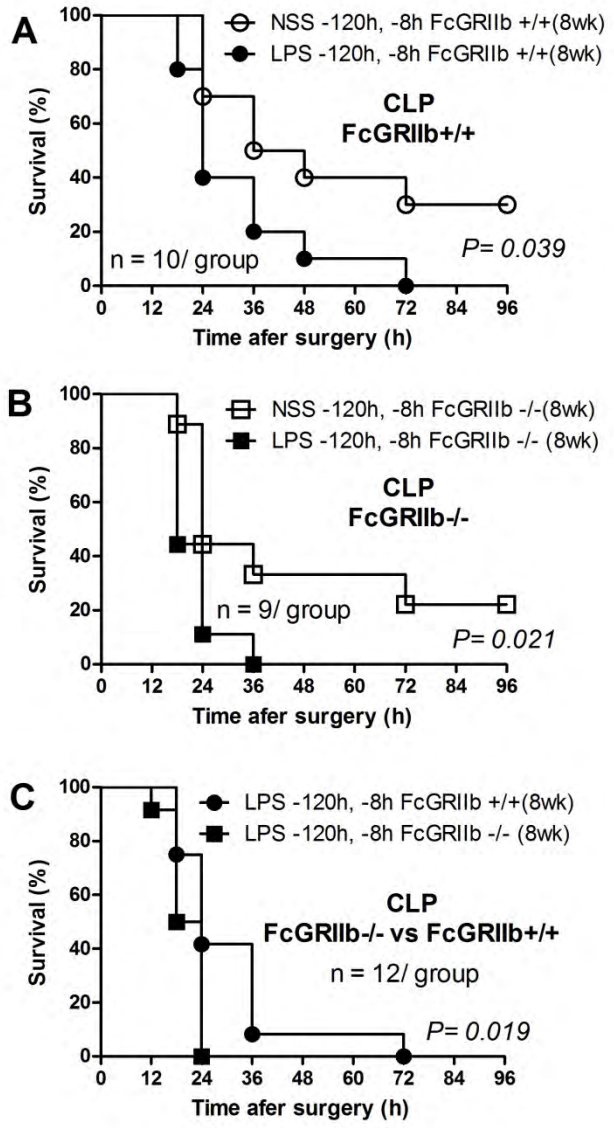

***Figure 3**. Survival analysis of cecal ligation and puncture (CLP) sepsis surgery preconditioning with 2 separated doses of LPS at 120h and 12h prior to CLP, CLP in endotoxin preconditioning model, in comparison with normal saline (NSS) placebo injection within wild type mice (FcGRIIb+/+) (A) and FcGRIIb-/- group (B) were showed. Survival analysis of CLP in endotoxin induced immunoparalysis model between wild type (FcGRIIb+/+) and FcGRIIb-/- mice (C) was also demonstrated.*

The cytokine responses after LPS injections and after superimposed by cecal ligation and puncture

The luminex-based multiplex system was used to explore the difference in the cytokines responses after LPS administration between asymptomatic FcGRIIb-/- mice versus wild type. Among the pro-inflammatory cytokines (TNF-α, IL-6, IL-1β and IFN-γ), we found that most of the pro-inflammatory cytokines, except for TNF-α, were significantly higher in FcGRIIb-/- mice at 1h after the 1st dose of LPS administration (Fig 4A-C). TNF-α, IL-6, IL-1β and IFN-γ at 1h after LPS administration in FcGRIIb-/- mice and wild type were 21,851±3,200, 3,525±117, 350±38, 21±2 pg/ml and 12,453±3,925, 2,301±157, 54±11, 3±1 pg/ml, respectively. In parallel, for the anti-inflammatory cytokines (IL-2, IL-4, IL-5 and IL-10), all of these cytokines, except for IL-5, were higher in FcGRIIb-/- mice at the 1st h after the 1st dose of LPS (Fig 4D-F). In detail, IL-2, IL-4, IL-5 and IL-10 at 1h after LPS administration in FcGRIIb-/- mice and wild type were 26.2±3.4, 10.8±1.1, 34.5±3.5, 958±106 pg/ml and 12.6±3.3, 3±0.4, 27.1±3.6, 575±104 pg/ml, respectively. These results supported the prominent cytokines responses in FcGRIIb-/- mice reported previously (18). Interestingly, at 1h and/or 2h of the 2nd LPS administration with the 5 times higher dose of LPS, all of these cytokines, except for IL-1β and IL-5, were significantly lower than the 1st administration in FcGRIIb-/- mice (Fig 4). In contrast, in wild type mice, only IFN-γ, IL-2, IL-4 and IL-10 were lower and IL-1β was higher in some time-point of 2nd LPS administration compared with the matched time-point of the 1st LPS injection (Fig 4). The endotoxin tolerance, determined by the lower cytokines responses after 2nd dose of LPS, was easier demonstrated in FcGRIIb-/- mice compare with wild type. Moreover, the severity of endotoxin tolerance, determined by the cytokine level difference after matched time-point of 1st and 2nd LPS administration, was higher in FcGRIIb-/- mice (Fig 4 inset graph).

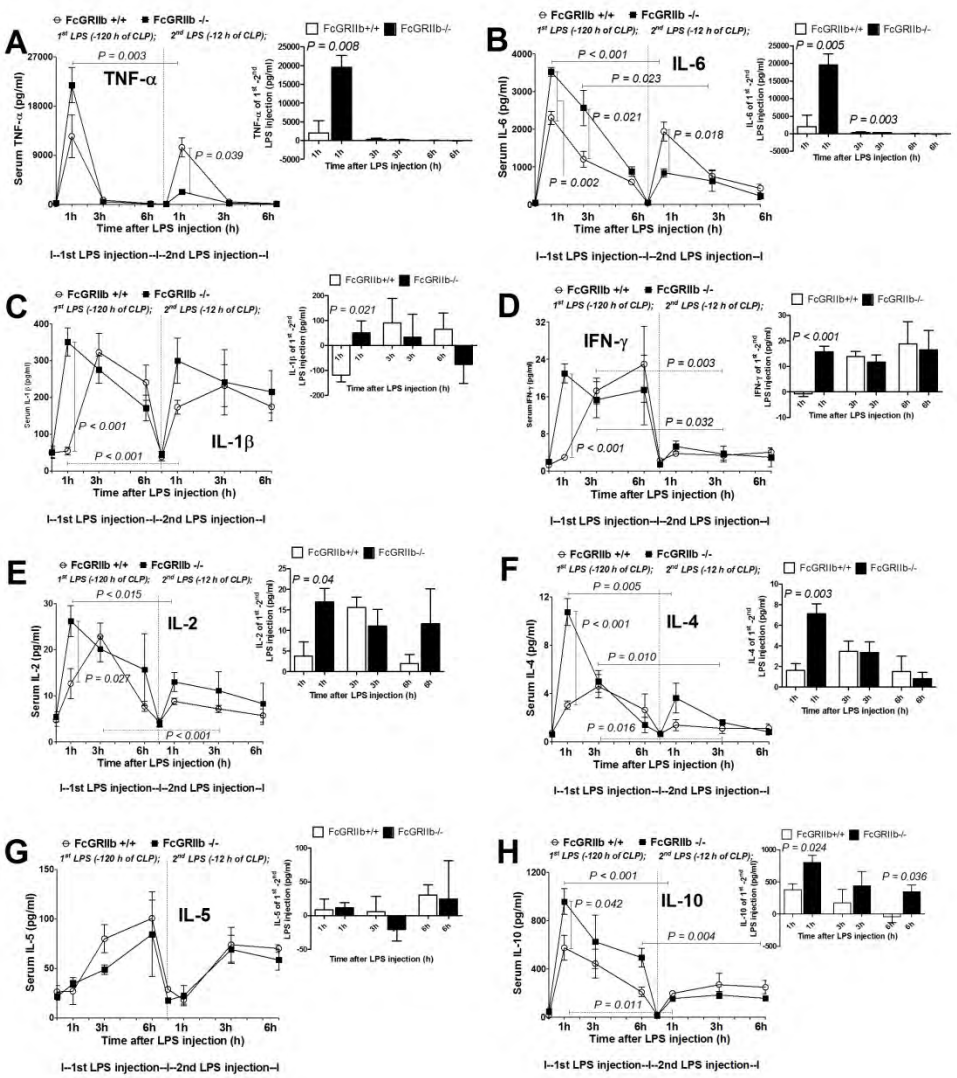

Figure 4. *Serum cytokines in wild type (FcGRIIb+/+) or FcGRIIb-/- mice after at 1, 3 and 6h after 1st LPS injection (0.8 g/kg) and 2nd LPS injection (4 g/kg) as measured by TNF-α (A), IL-6 (B), IL-1β (C), IFN-γ (D), IL-2 (E), IL-4 (F), IL-5 (G) and IL-10 (H) was demonstrated. To emphasize the difference of serum cytokines after 1st and 2nd doses of LPS, the delta change of serum cytokine response at the matched-time=points after both LPS injection was showed as inset graph. (n= 5-7 per group)*

It is interesting to note that, even with the 5 times higher LPS dose of 2nd LPS administration, most cytokines level was lower than the 1st dose implied endotoxin tolerance status in both FcGRIIb-/- and wild type mice. Subsequently, we tested the severity of polymicrobial infection in

these mice with CLP surgery and selected to explore only frequently mentioned sepsis cytokines (TNF-α, IL-6 and IL-10) *in vivo*.

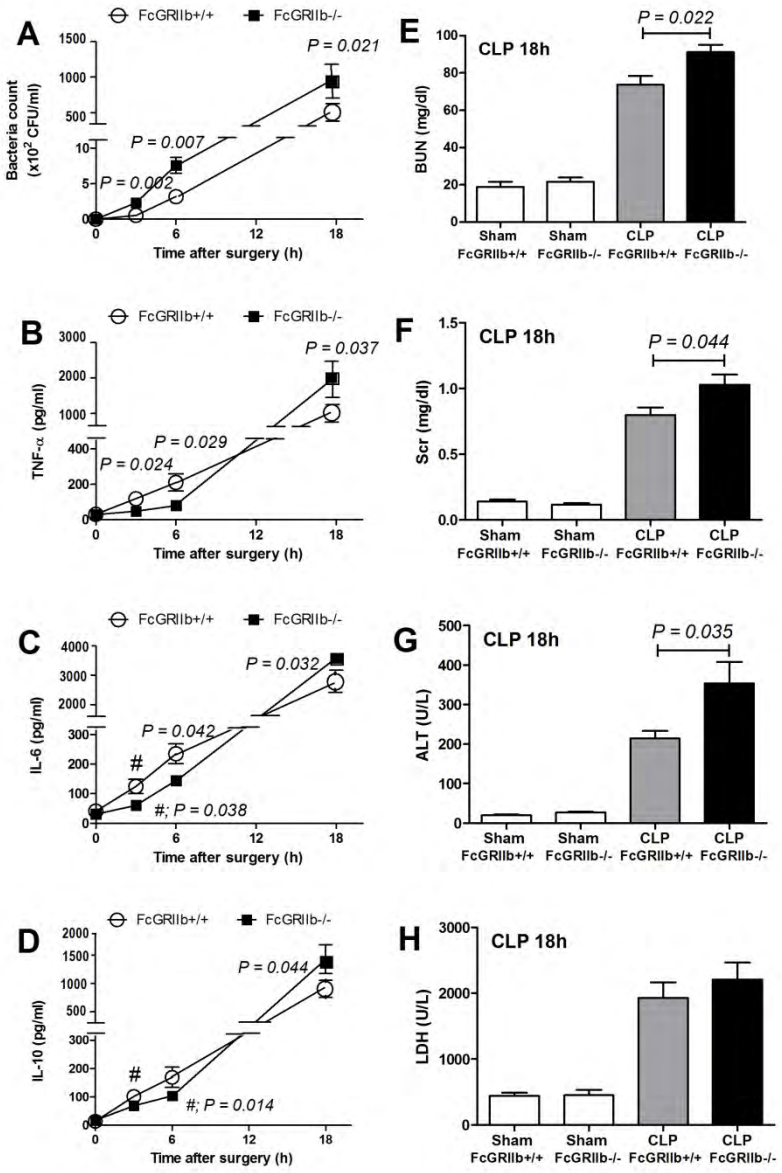

Figure 5. *The time-course of bacterial burdens after cecal ligation and puncture (CLP) in blood bacterial burdens (A), mononuclear cell (B), PMN (C), TNF-α (D), IL-6 (E), IL-10 (F) and the severity of organs injury after 18h of wild type (FcGRIIb+/+) and FcGRIIb-/- as demonstrated by serum creatinine (Scr) (G) and alanine transaminase (ALT) (H) was showed. (n= 5-7/ time-point and group)*

Interestingly, bacterial burdens in FcGRIIb-/- mice were higher than wild type in all selected time-points (3, 6 and 18h) after CLP (Fig 5A). Blood bacterial count ($x10^2$ CFU/ml) in FcGRIIb-/- mice and wild type at 3, 6 and 18h were 3.1±0.3, 7.6±1.1, 964±137 and 0.6±0.2, 3.3±0.6, 518±73, respectively.

Moreover, pro-inflammatory cytokines (TNF-α and IL-6) and anti-inflammatory cytokine (IL-10) were higher in wild type mice at 3 and 6h and only 3h after CLP, respectively (fig 5B-D). Serum TNF-α, IL-6 and IL-10 at 3h and 6h in FcGRIIb-/- versus wild type were 48±9, and 80±7.1, 69±9 and 145±18, 71±6 and 104±4 pg/ml versus 120±24 and 211±49, 124±24 and 235±33, 102±7 and 170±36 pg/ml, respectively. In contrast, at 18h after CLP, all of these cytokines and most of the organs injury biomarkers (Scr for kidney injury and ALT for liver injury) were higher in FcGRIIb-/- mice (Fig 5B-H). Despite the prominent responses to LPS in FcGRIIb-/- mice mentioned earlier, cytokines responses at the early phase of CLP after LPS preconditioning were stun in comparison with wild type implied the more severe immunoparalysis.

Perhaps, severe immunoparalysis at the early phase of sepsis might associate with the higher bacterial burdens leading to the higher sepsis severity (Fig 5) and mortality rate (Fig 3C) in FcGRIIb-/- mice.

Bone marrow derived macrophage of FcGRIIb-/- mice showed higher cytokine responses in the single incubation of LPS but lower responses in the double incubation of LPS

The previous results demonstrated that FcGRIIb-/- mice, an inhibitory signaling deficiency, showed a very high initial response, but subsequently follow with a more apparent exhaustion after LPS stimulation. Because macrophage might be responsible for the exhaustion *in vivo* then we tested FcGRIIb-/- macrophages response to endotoxin incubations *in vitro* in comparison with wild type cell.

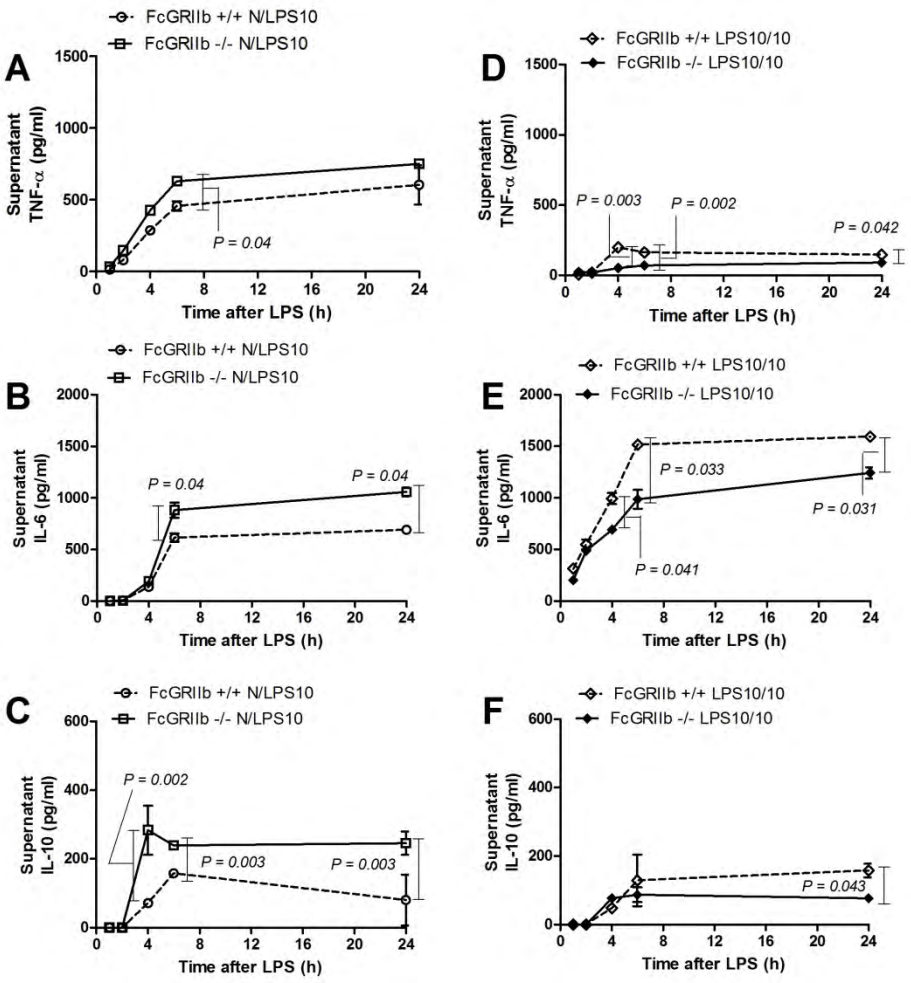

Figure 6. *The cytokine responses in supernatant media from macrophages of FcGRIIb-/- or wild type (FcGRIIb+/+) after activated with only once low dose LPS (N/LPS10) as measured by TNF-α (A), IL-6 (B), IL-10 (C) and after activated with twice low dose LPS (LPS10/10) as measured by TNF-α (D), IL-6 (E), IL-10 (F) was showed. (Separated experiments were done in triplicate)*

In parallel with the *in vivo* results, higher cytokines, at least in some time-points (3-24h), found in the supernatant of FcGRIIb-/- macrophages with the single low dose LPS (non LPS at the 1st 24h of the incubation followed by LPS dose at 10 μg/ml; N/LPS10) (Fig 6A-C). Then TNF-a and IL-10, but not IL-6, was lower in the double low dose of LPS stimulation (LPS 10 μg/ml for 24h then washed and add the same 2nd dose; LPS10/10) (Fig 6D-F). Then, a higher dose of LPS was used. Once again, FcGRIIb-/- macrophages showed the higher responses than wild type in the single high dose of LPS (N/LPS100) (Fig 7A-C). But the cytokines responses of macrophages primed with

the high dose of LPS seems to depend on doses of the 2nd LPS. In high LPS followed by low dose LPS (LPS100/10), all cytokines were detected at the low level (Fig 7D-F) and the difference between wild type and FcGRIIb-/- cells were subtle. But the apparent lower TNF-α and IL-10 in FcGRIIb-/- cells appeared again with the higher 2nd dose of LPS (LPS100/100) (Fig 7G-I). To clarify the macrophage exhaustion, the lower cytokines level after double LPS exposure compare with single LPS exposure, the cytokines level after single and double LPS exposure at 6 and 24h was demonstrated (Fig 8). With the double low dose of LPS (LPS10/10), macrophage exhaustion could be demonstrated with only the lower TNF-a in wild type cell but lower both TNF-a and IL-10 in FcGRIIb-/- macrophages (Fig 8A-C).

Interestingly, macrophage exhaustion, lower cytokine in double LPS exposure compare with single LPS, could be demonstrated only by TNF-a and IL-10 in FcGRIIb-/- cells but with TNF-a alone for wild type cell with the double low dose of LPS (LPS10/10) (Fig 8A-C). On the other hand, with the higher dose of LPS stimulation, the exhaustion could be demonstrated in all cytokines in FcGRIIb-/- cells but only in some condition in wild type cell (Fig 8D-F).

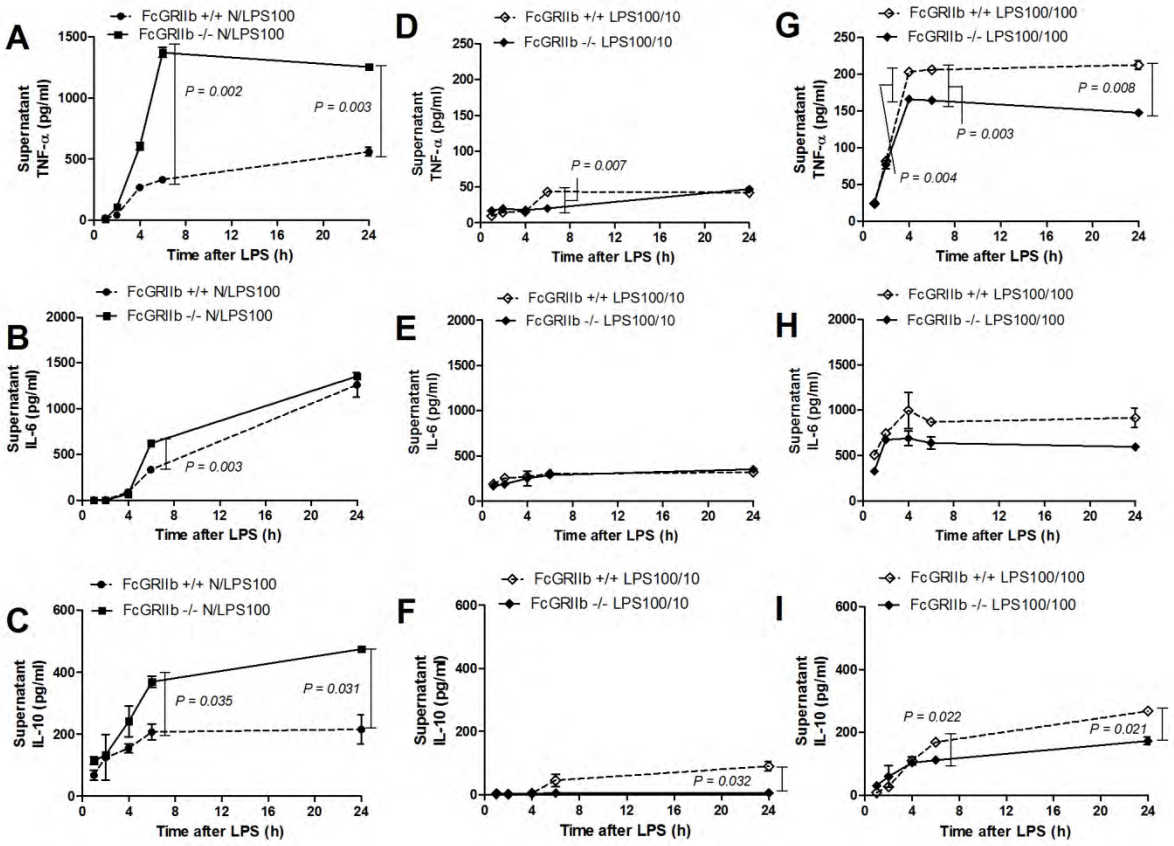

Figure 7. *The cytokine responses in supernatant media from macrophages of FcGRIIb-/- or wild type (FcGRIIb+/+) after activated with only once high dose LPS (N/LPS100) as measured by TNF-α, IL-6, IL-10 (A-C) and double LPS doses, low and high dose LPS (LPS100/10 and LPS100/100, respectively), (D-F) were demonstrated. (Separated experiments were done in triplicate)*

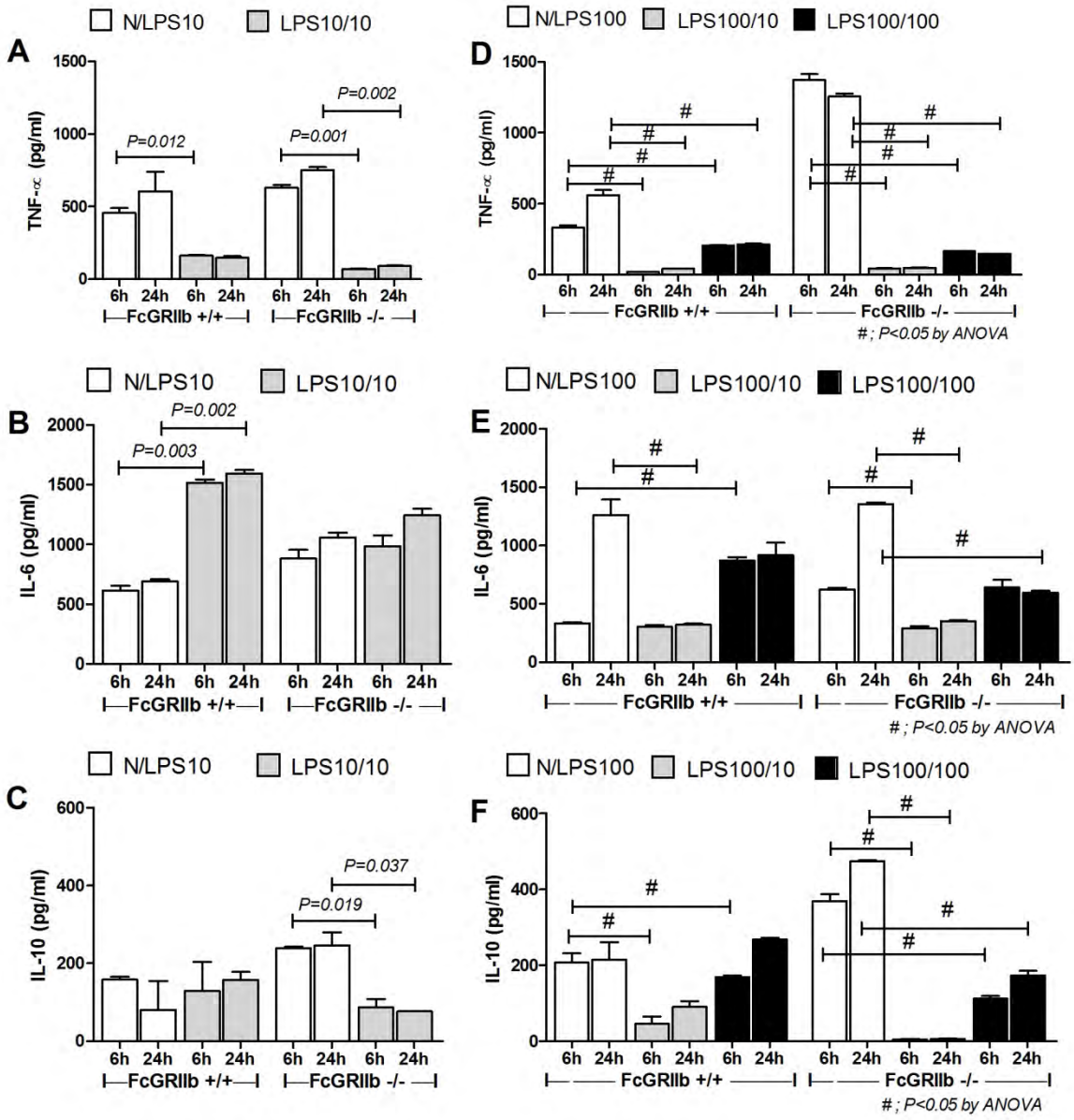

Figure 8. *The macrophage endotoxin tolerance emphasized by the difference in cytokine responses (TNF-α, IL-6, IL-10) in supernatant media from macrophages of FcGRIIb-/- or wild type (FcGRIIb+/+) after activated with only once low dose (N/LPS10) versus twice low dose of LPS (LPS10/10) (A-C) and the difference among only once high dose (N/LPS100) versus twice high and then low dose of LPS (LPS100/10) versus twice high dose of LPS (LPS100/100) (D-F) were demonstrated. (Separated experiments were done in triplicate)*

Macrophage exhaustion could not be demonstrated by IL-6 responses both in wild type and FcGRIIb-/- cells with double low dose LPS stimulation. In parallel, with the comparison among single high dose LPS (N/LPS100) with double LPS dose with low and high 2nd LPS dose (LPS100/10 and LPS100/100, respectively), macrophage exhaustion could be shown by all cytokines despite a less prominent in IL-6 response of the wild type cell (Fig 8D-F). It seems the macrophage exhaustion occur in both knock-out and wild type cells but more prominent in FcGRIIb-/- cells.

In contrast, the phagocytosis and killing activity of FcGRIIb -/- macrophages were better than wild type cells and there was a non-significant exhaustion in phagocytosis and killing activity after LPS stimulation (Fig 9). Although, the killing activity of FcGRIIb -/- macrophage should be able to control sepsis severity, perhaps the more severe bacterial sepsis severity in FcGRIIb -/- mice might due to the lower number of mononuclear cell after sepsis (Fig 5).

3. อภิปราย / วิจารณ์ (Discussion) ผลการทดลอง / ผลการวิจัยที่ได้ทั้งหมด (ทั้งที่เป็นและไม่เป็นไปตามสมมติฐานที่ตั้งไว้)

FcGRIIb deficiency is one of the genetic defects of SLE and FcGRIIb polymorphism with a lower gene activity commonly reported in patients with SLE in Asia (3-5, 8). Additionally, sepsis is the leading cause of death in patients with SLE (9). We showed a high susceptibility to CLP in symptomatic SLE mice but not in asymptomatic group in comparison with age-match wild type control. In asymptomatic genetic prone mice, the high susceptibility to CLP was showed only after with repeated endotoxin induced immunoparalysis. The more severe macrophage paralysis in FcGRIIb-/- macrophages might responsible for the more immunoparalysis in mice leading to higher sepsis severity after CLP surgery.

The susceptibility to bacterial infection in patients with SLE is associated with several factors; immunosuppressive drugs, activity of disease, organs involvement, etc (9). Studies on FcGRIIb-/- mice allow for exploring SLE without several confounding factors, especially immunosuppressive drugs. As expected, the higher mortality rate of sepsis was showed in 24-wk-old FcGRIIb-/- mice, positive proteinuria but normal Scr classified into symptomatic SLE group, in comparison with age-matched wild type mice. These results supported the association between SLE disease activity and bacterial infection susceptibility reported previously (9). Interestingly, the mortality rate of sepsis without LPS preconditioning in asymptomatic 8-wk-old FcGRIIb-/- mice,

positive anti-dsDNA without proteinuria, did not different to wild type mice supported the effective immune responses to bacterial infection previously published (18).

More prominent endotoxin-induced immunoparalysis in FcGRIIb-/- mice demonstrated by the high mortality rate of CLP sepsis

The immunoparalysis was induced by the double separate LPS administration and the severity of immunoparalysis, the condition with a more susceptible to infection, was determined by the severity of CLP sepsis (20). With this model, there was a higher mortality rate of CLP after LPS preconditioning compare with NSS placebo control within either 8-wk-old FcGRIIb-/- or wild type mice demonstrated immunoparalysis occur in both groups. Nevertheless, FcGRIIb-/- mice showed a higher mortality rate than wild type implied a more severe immunoparalysis.

Despite immunoparalysis could be demonstrated by several biomarkers (42), cytokines responses are frequently used. Then we tested cytokines responses in panels of pro- and anti-inflammatiory cytokines, TNF-α, IL-6, IL-1β, IFN-γ and IL-2, IL-4, IL-5, IL-10, respectively, with luminex-based measurement in mice with double doses of LPS administration. Most of the cytokines selected to measure were mainly produced by macrophage except for IFN-γ and IL-5 which produced prominently by NK cell or T cell and mast cell, respectively. With double dose of LPS administration by the 2nd dose of the 5 times higher than the 1st dose, all of these cytokines response after the 2nd LPS stimulation were not higher than the responses after the 1st LPS dose. These results suggested immunoparalysis in both FcGRIIb-/- and wild type mice. Interestingly, in comparison with wild type mice, most of the pro- and anti-inflammatory cytokins, except for IL-5, were higher in FcGRIIb-/- mice after the 1st LPS stimulation, implied the vigorous cytokines responses in FcGRIIb-/- mice. Then after 2nd LPS administration, all cytokine except for IL-1β and IL-5 were lower than the 1st responses in both wild type and but with a more prominent difference, demonstrated by the cytokine difference between 1st and 2nd LPS administration, in FcGRIIb-/- mice. These results demonstrated a more severe immunoparalysis in FcGRIIb-/- group. Of note, most of these cytokines produced from macrophages except for IL-5 and IFN-γ which produced mainly from mast cell and NK cell, respectively. Although functional FcGRIIb expression on mast cell (43) and murine NK cell (44) were reported, IFN-γ, but not IL-5, response differently between FcGRIIb-/-

and wild type mice. These implied the difference of FcGRIIb function between these cells. More experiments needed but out of the scope of this article.

Nevertheless, the double LPS preconditioning seems to affect CLP severity. At the initial time-point of CLP surgery, 12h after 2nd dose of LPS, there was non-difference in TNF-α, IL-6 and IL-10 between LPS preconditioning and NSS control (data not showed) supported by the base-line value before CLP surgery (Figure 5). Interestingly, blood bacterial burdens were higher with the lower of these cytokines in FcGRIIb-/- mice compare with wild type at 3h and 6h after CLP surgery. However, at 18h after CLP the sepsis severity was more severe in FcGRIIb-/- mice as demonstrated by bacterial burdens, cytokines and organs injury. Perhaps, the initial cytokine responses were needed for the initial innate immune responses to control the infection and the loss of the initial control in FcGRIIb-/- mice due to LPS preconditioning induced a more severe sepsis. These results support the importance of the initial bacterial control, especially in patients with SLE and /or FcGRIIb polymorphism.

More prominent immunoparalysis in FcGRIIb-/- macrophage demonstrated by cytokine responses after LPS stimulation

The LPS induction *in vitro* alters macrophage characteristics from classical pro-inflammatory macrophage responses into a less pro-inflammatory stage of the macrophage (38) implied the importance of macrophage in immunoparalysis. With the double low doses of LPS (LPS10/10), macrophage exhaustion could be demonstrated in both FcGRIIb-/- and wild type but cytokines production after 2nd dose of LPS was lower in FcGRIIb-/- macrophages. Moreover, the exhaustion seems to be more apparent with the higher 1st dose of LPS which needed the higher 2nd dose of LPS to re-stimulate. With the initial high dose of LPS followed by a low dose (LPS100/10), only subtle cytokines responses were demonstrated. But with the larger 2nd LPS dose (LPS100/100), the difference between FcGRIIb-/- and wild type appeared again. However, FcGRIIb-/- macrophages produced less cytokines levels after 2nd dose of LPS either with high or low LPS doses. Then LPS could induce a more apparent macrophage paralysis in FcGRIIb-/- cells resulted in immunoparalysis state in mice which demonstrated by the higher CLP sepsis severity.

In conclusion, we demonstrated the impact of the repeated infection in patients with SLE through the CLP preconditioning with LPS in FcGRIIb-/- mice. Despite a good response to the only once bacterial infection in FcGRIIb-/- mice, the responses to the repeated exposure might be impaired lead to a more severe bacterial burdens and infection. The repeated infection in patients with SLE could be more severe due not only to hyperimmunoglobulin induced hyper-immuneresponse and sepsis (18), but also from immunoparalysis with the higher bacterial burdens as currently demonstrated. In the translational aspect, we suggested that the repeated infection in patients with SLE should be vigorously concerned and the FcGRIIb polymorphism screening in Asian patients with SLE might be a useful clinical practice.

4. สรุปและเสนอแนะเกี่ยวกับการวิจัยในขั้นต่อไป ตลอดจนประโยชน์ในทางประยุกต์ของผลงานวิจัยที่ได้

The next final part of the project is the experiments to see if macrophage of FcGRIIb knock-out which demonstrated exhaustion but intact killing activity show shorter half-life in comparison with wild type cell. If the last experiment results go along with the hypothesis then FcGRIIb knock-out mice will be susceptible to sepsis due to macrophage exhaustion and shorten half life of macrophage which will be stronger support our in vivo results.

Regarding to the translation, the exploration of FcGR polymorphisms in patients with SLE might be beneficial for the prediction of sepsis and might require a more aggressive antibacterial drug at the beginning of the simple infectious diseases. More studies will be needed.

ส่วนประกอบตอนท้าย

1. บรรณานุกรม (Bibliography) ระบุรายชื่อเอกสารอ้างอิงโดยเรียงลำดับเอกสารอ้างอิงภาษาไทย ก่อนแล้วตามด้วยเอกสารภาษาต่างประเทศ ทั้งนี้ให้เรียงตามลำดับ

1. Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011 Dec 1;365(22):2110-21.

2. Crispin JC, Hedrich CM, Tsokos GC. Gene-function studies in systemic lupus erythematosus. Nat Rev Rheumatol. 2013 Aug;9(8):476-84.

3. Jakes RW, Bae SC, Louthrenoo W, Mok CC, Navarra SV, Kwon N. Systematic review of the epidemiology of systemic lupus erythematosus in the Asia-Pacific region: prevalence, incidence, clinical features, and mortality. Arthritis Care Res (Hoboken). 2012 Feb;64(2):159-68.

4. Siriboonrit U, Tsuchiya N, Sirikong M, Kyogoku C, Bejrachandra S, Suthipinittharm P, et al. Association of Fcgamma receptor IIb and IIIb polymorphisms with susceptibility to systemic lupus erythematosus in Thais. Tissue Antigens. 2003 May;61(5):374-83.

5. Tsuchiya N, Kyogoku C. Role of Fc gamma receptor IIb polymorphism in the genetic background of systemic lupus erythematosus: insights from Asia. Autoimmunity. 2005 Aug;38(5):347-52.

6. Bolland S, Ravetch JV. Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis. Immunity. 2000 Aug;13(2):277-85.

7. Clatworthy MR, Willcocks L, Urban B, Langhorne J, Williams TN, Peshu N, et al. Systemic lupus erythematosus-associated defects in the inhibitory receptor FcgammaRIIb reduce susceptibility to malaria. Proc Natl Acad Sci U S A. 2007 Apr 24;104(17):7169-74.

8. Chu ZT, Tsuchiya N, Kyogoku C, Ohashi J, Qian YP, Xu SB, et al. Association of Fcgamma receptor IIb polymorphism with susceptibility to systemic lupus erythematosus in Chinese: a common susceptibility gene in the Asian populations. Tissue Antigens. 2004 Jan;63(1):21-7.

9. Zandman-Goddard G, Shoenfeld Y. Infections and SLE. Autoimmunity. 2005 Nov;38(7):473-85.

10. Noel V, Lortholary O, Casassus P, Cohen P, Genereau T, Andre MH, et al. Risk factors and prognostic influence of infection in a single cohort of 87 adults with systemic lupus erythematosus. Ann Rheum Dis. 2001 Dec;60(12):1141-4.

11. Bosch X, Guilabert A, Pallares L, Cerveral R, Ramos-Casals M, Bove A, et al. Infections in systemic lupus erythematosus: a prospective and controlled study of 110 patients. Lupus. 2006;15(9):584-9.

12. Navarro-Zarza JE, Alvarez-Hernandez E, Casasola-Vargas JC, Estrada-Castro E, Burgos-Vargas R. Prevalence of community-acquired and nosocomial infections in hospitalized patients with systemic lupus erythematosus. Lupus. 2010 Jan;19(1):43-8.

13. Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, et al. Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. Medicine (Baltimore). 2003 Sep;82(5):299-308.

14. Ropes MW. Observations on the Natural Course of Disseminated Lupus Erythematosus. Medicine (Baltimore). 1964 May;43:387-91.

15. Radaev S, Sun P. Recognition of immunoglobulins by Fcgamma receptors. Mol Immunol. 2002 May;38(14):1073-83.

16. Nimmerjahn F, Ravetch JV. Fcgamma receptors: old friends and new family members. Immunity. 2006 Jan;24(1):19-28.

17. Pinheiro da Silva F, Aloulou M, Skurnik D, Benhamou M, Andremont A, Velasco IT, et al. CD16 promotes Escherichia coli sepsis through an FcR gamma inhibitory pathway that prevents phagocytosis and facilitates inflammation. Nat Med. 2007 Nov;13(11):1368-74.

18. Clatworthy MR, Smith KG. FcgammaRIIb balances efficient pathogen clearance and the cytokine-mediated consequences of sepsis. J Exp Med. 2004 Mar 1;199(5):717-23.

19. Maglione PJ, Xu J, Casadevall A, Chan J. Fc gamma receptors regulate immune activation and susceptibility during Mycobacterium tuberculosis infection. J Immunol. 2008 Mar 1;180(5):3329-38.

20. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol. 2013 Dec;13(12):862-74.

21. Hotchkiss RS, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. Lancet Infect Dis. 2013 Mar;13(3):260-8.

22. Davis CG, Chang K, Osborne D, Walton AH, Dunne WM, Muenzer JT. Increased susceptibility to Candida infection following cecal ligation and puncture. Biochem Biophys Res Commun. 2011 Oct 14;414(1):37-43.

23. Jung E, Perrone EE, Liang Z, Breed ER, Dominguez JA, Clark AT, et al. Cecal ligation and puncture followed by methicillin-resistant Staphylococcus aureus pneumonia increases mortality in mice and blunts production of local and systemic cytokines. Shock. 2012 Jan;37(1):85-94.

24. Leelahavanichkul A, Somparn P, Bootprapan T, Tu H, Tangtanatakul P, Nuengjumnong R, et al. High-dose ascorbate with low-dose amphotericin B attenuates severity of disease in a model of re-appearance of candidemia during sepsis in the mouse. Am J Physiol Regul Integr Comp Physiol. 2015 May 20:ajpregu 00238 2014.

25. Unsinger J, Burnham CA, McDonough J, Morre M, Prakash PS, Caldwell CC, et al. Interleukin-7 ameliorates immune dysfunction and improves survival in a 2-hit model of fungal sepsis. J Infect Dis. 2012 Aug 15;206(4):606-16.

26. Leentjens J, Kox M, Koch RM, Preijers F, Joosten LA, van der Hoeven JG, et al. Reversal of immunoparalysis in humans in vivo: a double-blind, placebo-controlled, randomized pilot study. Am J Respir Crit Care Med. 2012 Nov 1;186(9):838-45.

27. Shi DW, Zhang J, Jiang HN, Tong CY, Gu GR, Ji Y, et al. LPS pretreatment ameliorates multiple organ injuries and improves survival in a murine model of polymicrobial sepsis. Inflamm Res. 2011 Sep;60(9):841-9.

28. Wheeler DS, Lahni PM, Denenberg AG, Poynter SE, Wong HR, Cook JA, et al. Induction of endotoxin tolerance enhances bacterial clearance and survival in murine polymicrobial sepsis. Shock. 2008 Sep;30(3):267-73.

29. Leelahavanichkul A, Huang Y, Hu X, Zhou H, Tsuji T, Chen R, et al. Chronic kidney disease worsens sepsis and sepsis-induced acute kidney injury by releasing High Mobility Group Box Protein-1. Kidney Int. 2011 Dec;80(11):1198-211.

30. Boomer JS, Green JM, Hotchkiss RS. The changing immune system in sepsis: is individualized immuno-modulatory therapy the answer? Virulence. 2014 Jan 1;5(1):45-56.

31. Lopez-Collazo E, del Fresno C. Pathophysiology of endotoxin tolerance: mechanisms and clinical consequences. Crit Care. 2013;17(6):242.

32. Morris MC, Gilliam EA, Li L. Innate immune programing by endotoxin and its pathological consequences. Front Immunol. 2014;5:680.

33. Ravetch JV, Bolland S. IgG Fc receptors. Annu Rev Immunol. 2001;19:275-90.

34. Kox M, de Kleijn S, Pompe JC, Ramakers BP, Netea MG, van der Hoeven JG, et al. Differential ex vivo and in vivo endotoxin tolerance kinetics following human endotoxemia. Crit Care Med. 2011 Aug;39(8):1866-70.

35. Draisma A, Pickkers P, Bouw MP, van der Hoeven JG. Development of endotoxin tolerance in humans in vivo. Crit Care Med. 2009 Apr;37(4):1261-7.

36. Mihara M, Tan I, Chuzhin Y, Reddy B, Budhai L, Holzer A, et al. CTLA4Ig inhibits T cell-dependent B-cell maturation in murine systemic lupus erythematosus. J Clin Invest. 2000 Jul;106(1):91-101.

37. Boonyatecha N, Sangphech N, Wongchana W, Kueanjinda P, Palaga T. Involvement of Notch signaling pathway in regulating IL-12 expression via c-Rel in activated macrophages. Mol Immunol. 2012 Jul;51(3-4):255-62.

38. Pena OM, Pistolic J, Raj D, Fjell CD, Hancock RE. Endotoxin tolerance represents a distinctive state of alternative polarization (M2) in human mononuclear cells. J Immunol. 2011 Jun 15;186(12):7243-54.

39. Kraatz J, Clair L, Rodriguez JL, West MA. In vitro macrophage endotoxin tolerance: defective in vitro macrophage map kinase signal transduction after LPS pretreatment is not present in macrophages from C3H/HeJ endotoxin resistant mice. Shock. 1999 Jan;11(1):58-63.

40. Lee HS, Lee GS, Kim SH, Kim HK, Suk DH, Lee DS. Anti-oxidizing effect of the dichloromethane and hexane fractions from Orostachys japonicus in LPS-stimulated RAW 264.7 cells via upregulation of Nrf2 expression and activation of MAPK signaling pathway. BMB Rep. 2014 Feb;47(2):98-103.

41. Vonk AG, Wieland CW, Netea MG, Kullberg BJ. Phagocytosis and intracellular killing of Candida albicans blastoconidia by neutrophils and macrophages: a comparison of different microbiological test systems. J Microbiol Methods. 2002 Mar;49(1):55-62.

42. Hamers L, Kox M, Pickkers P. Sepsis-induced immunoparalysis: mechanisms, markers, and treatment options. Minerva Anestesiol. 2015 Apr;81(4):426-39.

43. Tkaczyk C, Okayama Y, Metcalfe DD, Gilfillan AM. Fcgamma receptors on mast cells: activatory and inhibitory regulation of mediator release. Int Arch Allergy Immunol. 2004 Mar;133(3):305-15.

44. Perussia B, Tutt MM, Qiu WQ, Kuziel WA, Tucker PW, Trinchieri G, et al. Murine natural killer cells express functional Fc gamma receptor II encoded by the Fc gamma R alpha gene. J Exp Med. 1989 Jul 1;170(1):73-86.

2. ภาคผนวก (Appendix) ถ้ามี

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Publications:

1. **Leelahavanichkul A**, Areepium N, Vadcharavivad S, Praditpornsilpa K, Avihingsanon Y, Karnjanabuchmd T, Eiam-Ong S and Tungsanga K. Pharmacokinetics of Sirolimus in Thai Healthy Volunteers. *J Med Assoc Thai* 2005: **88** Suppl 4: S157-62.
2. Nurnuansuwan S, **Leelahavanichkul A**, Pansin P and Eiam-Ong S. Intravenous iron administration during the maintenance period in erythropoietin-treated hemodialysis patients: a simple and effective method. *J Med Assoc Thai* 2005: **88** Suppl 4: S184-8.
3. Kanjanabuch T, Kittikovit W, Lewsuwan S, **Leelahavanichkul A**, Avihingsanon Y, Praditpornsilpa K, Eiam-Ong S, Tungsanga K and Sitprija V. Etiologies of glomerular diseases in Thailand: a renal biopsy study of 506 cases. *J Med Assoc Thai* 2005: **88** Suppl 4: S305-11.
4. Pichaiwong W, **Leelahavanichkul A** and Eiam-ong S. Efficacy of cellulose triacetate dialyzer and polysulfone synthetic hemofilter for continuous venovenous hemofiltration in acute renal failure. *J Med Assoc Thai* 2006: **89** Suppl 2: S65-72.

5. Dear JW, **Leelahavanichkul A**, Aponte A, Hu X, Constant SL, Hewitt SM, Yuen PS and Star RA. Liver proteomics for therapeutic drug discovery: inhibition of the cyclophilin receptor CD147 attenuates sepsis-induced acute renal failure. *Crit Care Med* 2007 Oct; **35**(10):2319-28. *(impact factor 6.5)*

6. Yasuda H*, **Leelahavanichkul A***, Tsunoda S, Dear JW, Takahashi Y, Ito S, Hu X, Zhou H, Doi K, Childs RW, Klinman DM, Yuen PS and Star RA. Chloroquine and inhibition of Toll-like receptor 9 protect from sepsis-induced acute kidney injury. *Am J Physiol Renal Physiol*. 2008 May; **294**(5):F1050-8. **(* distribution equally)** *(impact factor 4.6-5)*

7. Tiranathanagul K, Katavetin P, Injan P, **Leelahavanichkul A**, Techawathanawanna N, Praditpornsilpa K, Srisawat N, Tungsanga K and Eiam-Ong S. A novel simple hemoglobin dilution technique to measure hemodialysis vascular access flow. *Kidney Int*. 2008 May; **73**(9):1082-6. *(impact factor 6.4)*

8. Doi K, Hu X, Yuen PST, **Leelahavanichkul A**, Yasuda H, Kim SM, Schnermann J, Jonassen T, Frokiar, Nielson S and Star RA. AP 214, an analogue of α-melanocyte-stimulating hormone, ameliorate sepsis induced acute kidney injury and mortality. *Kidney Int.*2008 Jun; **73**(11):1266-74. *(impact factor 6.4)*

9. Zhou H, Cheruvanky A, Hu X, Matsumoto T, Hiramatsu N, Cho ME, Berger A, **Leelahavanichkul A**, Doi K, Chawla LS, Illei GG, Kopp JB, Balow JE, Austin HA, Yuen PS, and Star RA. Urinary exosomal transcription factors, a new class of biomarkers for renal disease. *Kidney Int.* 2008 Sep; **74**(5):613-21. *(impact factor 6.4)*

10. Doi K, **Leelahavanichkul A**, Hu X, Yuen PS and Star RA. Pre-existing renal disease promotes sepsis-induced acute kidney injury and worsens outcome. *Kidney Int*. 2008 Oct; **74**(8):1017-25. *(impact factor 6.4)*

11. **Leelahavanichkul A**, Yasuda H, Doi K, Yuen PS, Hu X, Zhou H, Yuen PS and Star RA. Methyl-2-acetamidoacrylate decrease sepsis induced acute kidney injury in CD-1 mice. *Am J Physiol Renal Physiol.* 2008 Dec; **295**(6):F1825-35. *(impact factor 4.6-5)*

12. Nemeth K*, Leelahavanichkul A *, Yuen PS, Mayer B, Parmelee B, Doi K, Robey P.G., Leelahavanichkul K, Hu X, Star RA and Mezey E. Bone marrow stromal cells significantly attenuate mouse poly-microbial sepsis via monocyte/macrophage derived IL-10. *Nature Medicine*. 2009 Jan; **15**(1):42-9. **(* distribution equally)** *(impact factor 27.1)*

13. Doi K, Yuen PS, Eisner C, Hu X, Leelahavanichkul A, Schnermann J, Star RA. Reduced production of creatinine limits its use as marker of kidney injury in sepsis. *J Am Soc Nephrol*. 2009 Jun; **20**(6):1217-21. *(impact factor 7.1)*

14. Doi K, Leelahavanichkul A, Yuen PS, Star RA. Animal models of sepsis and sepsis- induced kidney injury (review). *J Clin Invest* 2009 Oct; **119**(10):2868-78. *(impact factor 16)*

15. Eisner C, Faulhaber-Walter R, Wang Y, Leelahavanichkul A, Yuen PS, Mizel D, Star RA, Briggs JP, Levine M, Schnermann J. Major contribution of tubular secretion to creatinine clearance in mice. *Kidney Int* 2010 Mar; **77**(6):519-26. *(impact factor 6.4)*

16. Leelahavanichkul A, Yan Q, Hu X, Eisner C, Huang Y, Chen R, Mizel D, Zhou H, Wright EC, Kopp JB, Schnermann J, Yuen PS, Star RA. Angiotensin II overcomes strain-dependent resistance of rapid CKD progression in a new remnant kidney mouse model. *Kidney Int* 2010 Dec; **78**(11): 1136-53. *(impact factor 6.4)*

17. Leelahavanichkul A, Huang Y, Hu X, Zhou H, Tsuji T, Kopp JB., Schnermann J, Yuen ST, Star RA. Chronic kidney disease-induced splenic apoptosis and HMGB1 elevation worsens sepsis and sepsis-induced acute kidney injury *Kidney Int* 2011; Dec; **80**(11):1198-211. *(impact factor 6.4)*

18. Wontanatawatot W, Eiam-Ong S, Leelahavanichkul A, Kanjanabuch T. An update on RAAS blockade and peritoneal membrane preservative: the ace of art. *J Med Assoc Thai* 2011 Sep; **94** (Suppl 4):S175-83.

19. Kanjanabuch T, Puttipittayathorn N, Leelahavanichkul A, Lieusuwan S, Katavetin P, Mahatanan N, Sriudom K, Chirananthavat T, Thongbor N, Eiam-Ong S. Exfoliated mesothelial cell and CA-125 in automated peritoneal dialysis (APD) and continuous ambulatory peritoneal dialysis (CAPD) patients. *J Med Assoc Thai* 2011 Sep; **94**(Suppl 4):S119-25.

20. Tiranathanagul K, Jearnsujitwimol O, Susantitaphong P, Kijkriengkraikul N, **Leelahavanichkul A**, Srisawat N, Praditpornsilpa K, Eiam-Ong S. Regional citrate anticoagulation reduces polymorphonuclear cell degranulation in critically ill patients treated with continuous venovenous hemofiltration. *Ther Apher Dial.* 2011 Dec; **15**(6):556-64. *(impact factor 1.1)*

21. Baranova IN, Vishnyakova TG, Bocharov AV, **Leelahavanichkul A**, Kurlander R, Chen Z, Souza AC, Yuen PS, Star RA, Csako G, Patterson AP, Eggerman TL. Class B Scavenger Receptor Types I and II and CD36 Mediate Bacterial Recognition and Proinflammatory Signaling Induced by Escherichia coli, Lipopolysaccharide, and Cytosolic Chaperonin 60. *J Immunol.* 2012 Feb 1;**188**(3):1371-80. *(impact factor 5.74)*

22. Zafrani L, Gerotziafas G, Byrnes C, Hu X, Perez J, Lévi C, Placier S, Letavernier E, **Leelahavanichkul A**, Haymann JP, Elalamy I, Miller JL, Star RA, Yuen PS, Baud L. Calpastatin improves survival and limits intravascular coagulopathy in polymicrobial sepsis : Importance of circulating microparticles. *Am J Respir Crit Care Med.* 2012 Apr 1;**185**(7):744-55. *(impact factor 10.19)*

23. **Leelahavanichkul A**, Bocharov AV, Kurlander R, Baranova IN, Vishnyakova TG, Hu X, Doi K, Csako G, Yuen ST, Star RA, Eggerman TL. Class B Scavenger Receptor Types I and II and CD36 Targeting Improves Sepsis Survival and Acute Outcomes in Mice. *J Immunol.* 2012 Mar 15;**188**(6):2749-58. *(impact factor 5.74)*

24. Somparn P, Hirankarn N, **Leelahavanichkul A**, Khovidhunkit W, Thongboonkerd V, Avihingsanon Y. Urinary proteomics revealed prostaglandin H_2D-isomerase, not Zn-α2-glycoprotein, as a biomarker for active lupus nephritis. *J Proteomics.* 2012 Jun 18; 75(11):3240-7 *(impact factor 4.87)*

25. Seujange Y*, **Leelahavanichkul A***, Yisarakun W, Khawsuk W, Meepool A, Phamonleatmongkol P, Saechau W, Onlamul W, Tantiwarattanatikul P, Oonsook W, Eiam-Ong S, Eiam-Ong S. Hibiscus Sabdariffa Linnaeus Aqueous Extracts Attenuate the Progression of Renal Injury in 5/6 Nephrectomy Rats. *Ren Fail.* 2012 Nov 19 **(* distribution equally)** *(impact factor 0.87)*

26. Treamtrakanpon W, Tantivitayakul P, Benjachat T, Somparn P, Kittikowit W, Eiam-ong S, **Leelahavanichkul A**, Hirankarn N and Avihingsanon Y. APRIL, A Proliferation-inducing Ligand, Can be Used to Predict the Prognosis of Lupus Nephritis. *Arthritis Research Ther*. 2012 Nov 21 (*impact factor 4.45*)

27. Zhou H, Hu X, Kajiyama H, Sakairi T, **Leelahavanichkul A**, Tsuji T, Vento S, Frank R, Kopp J, Trachtman H, Yuen ST, and Star RA. Urinary exosomal wilm's tumor-1 as potential biomarker for podocyte injury. *Am J Physiol Renal Physiol.* 2013 Aug15;305(4):F553-9. *(impact factor 3.79)*

28. Chancharoenthana W, Tiranathanagul K, Srisawat N, Susantitaphong P, **Leelahavanichkul A**, Praditpornsilpa K, Tungsanga K, Eiam-Ong S. Enhanced vascular endothelial growth factor and inflammatory cytokine removal with online hemodiafiltration over high-flux hemodialysis in sepsis-related acute kidney injury patients. *Ther Apher Dial*. 2013 Oct;17(5):557-63. *(impact factor 3.79)*

29. **Leelahavanichkul A**, Sauza A, Hsu V, Zhou H, Eisner C, Li L, Hu X, Tsuji T, Kent D, Schnermann J, Yuen ST, Star RA. Serum Cystatin C as the marker of sepsis induce acute kidney injury in CD-1 mice; the usefulness and the limitation. *Am J Physiol Renal Physiol.* 2014 Aug; F939-F948. *(impact factor 3.79)*

30. Chancharoenthana W, Townamchai N, Pongpirul K, Kittiskulnam P,**Leelahavanichkul A**, Avihingsanon Y, Suankratay C, Wattanatorn S, Kittikowit W, Praditpornsilpa K, Tungsanga K, Eiam-Ong S. The Outcomes of Kidney Transplantation in Hepatitis B Surface Antigen (HBsAg)-Negative Recipients Receiving Graft From HBsAg-Positive Donors: A Retrospective, Propensity Score-Matched Study. *Am J Transplant*. 2014 Dec;14 (12):2814-20. *(impact factor 5.68)*

31. Huebner AR, Somparn P, Benjachat T, **Leelahavanichkul A**, Avihingsanon Y, Fenton RA, Pisitkun T. Exosomes in urine biomarker discovery. *Adv Exp Med Biol*. 2015;845:43-58. *(impact factor 3.79)*

32. **Leelahavanichkul A**, Somparn P, Panich T, Chancharoenthana W, Wongphom J, Pisitkun T, Hirankarn N, Eiam-Ong S. The levels of serum microRNA-122 in acute liver injury induced by

kidney injury and sepsis in CD-1 mouse models. *Hepatol Res*. 2015 Feb 5 (Epub). *(impact factor 1.96)*

33. Worasilchai N*, **Leelahavanichkul A***, Kanjanabuch T, Thongbor N, Lorvinitnun P, Sukhontasing K, Finkelman M, Chindamporn A. (1⟶3)-β-D-glucan and galactomannan testing for the diagnosis of fungal peritonitis in peritoneal dialysis patients, a pilot study. *Med Mycol*. 2015 May 1;53(4):338-46. **(* distribution equally)** *(impact factor 2.34)*

34. Supawanpen C, Nouanthong P, Jaruvongvanich V, Pongpirul K, Amornimit-Pongpirul W, **Leelahavanichkul A**, Kanjanabuch T. Urinary podocalyxin, the novel biomarker for detection of renal change in obesity. *J Nephrol*. 2015 April 15 (Epub) *(impact factor 1.45)*

35. **Leelahavanichkul A**, Somparn P, Bootprapan T, Tu H, Tangtanatakul P, Nuengjumnong R, Worasilchai N, Tiranathanagul K, Eiam-Ong S, Levine M, Chinampon A, Srisawat N. High-dose ascorbate with low-dose amphotericin B attenuates severity of disease in a model of re-appearance of candidemia during sepsis in the mouse. *Am J Physiol Regul Integr Comp Physiol*. 2015 Aug 1; 309(3): R223-34 *(impact factor 3.66)*

36. Benjachat T, Tongyoo P, Tantivitayakul P, Somparn P, Hirankarn N, Prom-On S,Pisitkun P, **Leelahavanichkul A**, Avihingsanon Y, Townamchai N. Biomarkers for Refractory Lupus Nephritis: A Microarray Study of Kidney Tissue. *Int J Mol Sci*.2015 Jun 23;16 (6):14276-90. *(impact factor 2.98)*

37. Tantivitayakul P, Benjachat T, Somparn P, **Leelahavanichkul A**, Kittikovit V, Hirankarn N, Pisitkun T, Avihingsanon Y. Elevated expressions of myeloid-related proteins-8 and -14 are danger biomarkers for lupus nephritis. Lupus. 2015 Jul 29. *(impact factor 2.6)*

38. **Leelahavanichkul A**, Pongpirul K, Thongbor N, Worasilchai N, Petphuak K, Thongsawang B, Towannang P, Lorvinitnun P, Sukhontasing K, Katavetin P, Praditpornsilpa K, Eiam-Ong S, Chindamporn A, Kanjanabuch T. (1⟶3)-β-D-glucan and galactomannan for a differentiation of chemical “black particles” and fungal particles inside peritoneal dialysis tubing. Perit Dial Int. 2015 Nov 2 *(impact factor 2.2)*

39. Townamchai N, Chancharoenthana W, Vadcharavivad S, Chariyavilaskul P, Pongpirul K, **Leelahavanichkul A**, Watanatorn S, Avihingsanon Y, Praditpornsilpa K, Srisawat N. A Simple Novel Technique to Estimate Tacrolimus Dosages During the Early Post Kidney Transplantation Period. Transplant Proc. 2015 Oct; 47(8):2433-8. *(impact factor 0.98)*

40. **Leelahavanichkul A**, Somparn P, Issara-Amphorn J, Eiam-ong S, Avihingsanon Y, Hirankarn N, Srisawat N. Advantage of serum neutrophil gelatinase associated lipocalin in sepsis, impact of bilateral nephrectomy and bilateral ureter obstruction mouse models. Shock. Accepted *(impact factor 3.2)*

41. **Leelahavanichkul A**, Worasilchai N, Wannalerdsakun S, Jutivorakool K, Somparn P Issara-Amphorn J,, Finkelman M, Chinamporn A.Serum (1⟶3)-β-D-glucan as a biomarker of gastrointestinal leakage in severe bacterial sepsis: murine endotoxin and cecal ligation/puncture models and a pilot study in patients with febrile neutropenia and sepsis. Shock. On revision *(impact factor 3.2)*