Oral presentation 1

時　間：113 年 12 月 14 日(星期六) 08：10-08：40

地　點：新竹喜來登大飯店 3 樓梅花桐花百合廳

座長/Moderator	高雄榮民總醫院 曾瑞成 醫師
08:10 - 08:22	**The B cell differentiation in the spleen of the imiquimod (IMQ) stimulated lupus FVB/N mouse model** Yu-Jih Su[1,2] [1]Internal medicine, Kaohsiung Chang-Gung Memorial Hospital, Taiwan ROC [1]Institute of Medical Science and Technology, [2]Institute of Biomedical Sciences, National Sun Yat-sen University, Taiwan ROC.
08:22 -8:25	Q & A
08:25 - 08:37	**Mechanisms of anti-dsDNA antibody production induced by commensal Streptococcus *mutans*: A study of molecular mimicry and NET formation in systemic lupus erythematosus** Yu-Min Kuo[1,4], , Zhi-Yun Lai[1], Chih-Chieh Hsu[3], Song-Chou Hsieh[1] , Chiau-Jing Jung[2] *[1]National Taiwan University College of Medicine and National Taiwan University Hospital, Division of Allergy, Immunology and Rheumatology, Department of Internal Medicine, Taipei, Taiwan, Republic of China* *[2]College of Medicine, Taipei Medical University, Microbiology and Immunology, School of Medicine, Taipei, Taiwan, Republic of China* *[3]National Taiwan University College of Medicine and National Taiwan University Hospital, Division of Infection, Department of Internal Medicine, Taipei, Taiwan, Republic of China* *[4]College of Medicine, National Taiwan University, Graduate Institute of Clinical Medicine, Taipei, Taiwan, Republic of China* **共生鏈球菌 S. *mutans* 誘導的抗雙股 DNA 抗體產生機制：釐清共生鏈球菌於紅斑狼瘡之致病角色** 郭佑民 [1,4] 賴芷昀 [1] 許智傑 [3] 謝松洲 [1] 鍾筱菁 [4] 1. 國立臺大醫學院附設醫院 內科部 過敏免疫風濕科 2. 國立臺灣大學醫學院臨床醫學研究所 3. 國立臺灣大學醫學院醫學院內科部 感染科 4. 台北醫學大學微生物免疫學科
08:37 -8:40	Q & A

The B cell differentiation in the spleen of the imiquimod (IMQ) stimulated lupus FVB/N mouse model.

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Background: Generation of autoantibodies and systemic inflammation are characteristic of dysregulation of B cells in the pathogenesis of lupus. Studying the population of B cells in the spleen can identify crucial B cell populations and provides potential therapeutic targets.
Methods: Lupus mouse model was established, and B cells were purified from spleen. Single-cell RNA sequencing was performed to identify different B cell clusters between study and control mice.
Results: The workflow of constructing SLE mouse model and the different B cell population in spleen are demonstrated in Figure 1. In this study, we identified 11 types of B cell populations. Pathways and transcriptional factors analysis of the interested clusters and expression profiles were demonstrated, comparing between study and control mice.
Conclusion(s): The splenocytes of lupus mouse exhibited an altered expression profile, predominantly involving genes related to inflammation and antibody production, which are likely to contribute to lupus pathogenesis. These findings provide new insights into the specific functions of B cell populations in lupus and reveal potential therapeutic targets for this autoimmune disease.

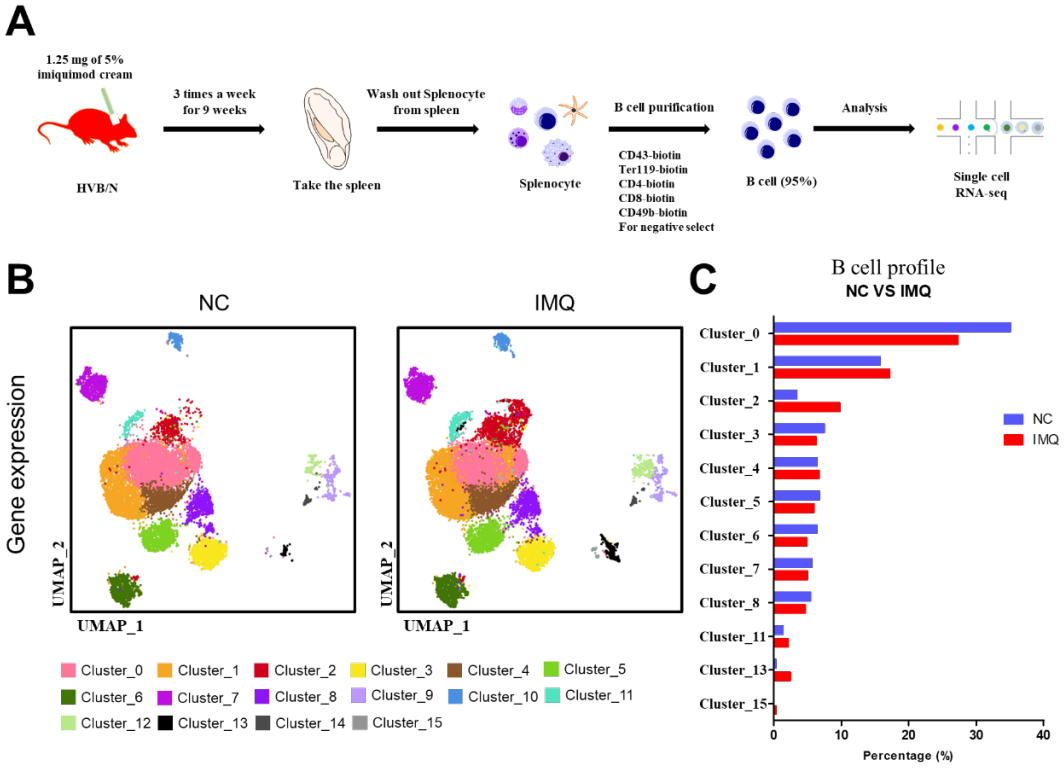
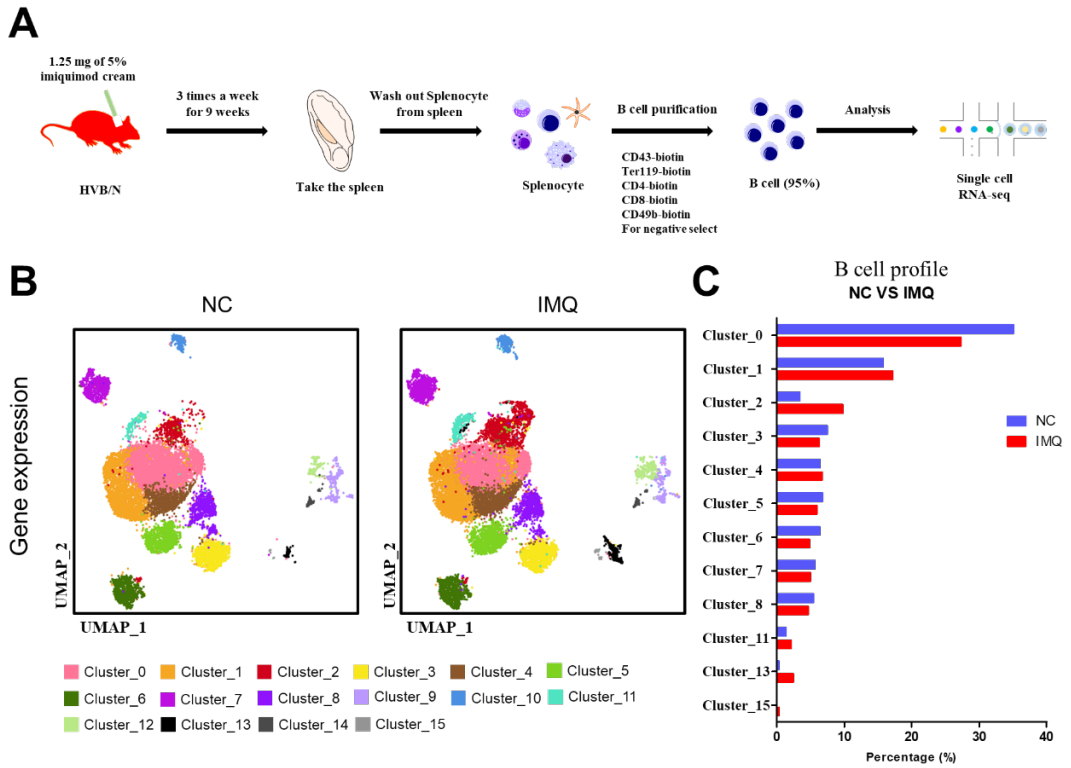


Fig. 1 The workflow of constructing SLE mouse model and the different B cell population in spleen. (A) Experimental design-construction of SLE mose model and purification of B cells in spleen for single-cell RNA-sequencing (sc-RNA-seq). (B) The Uniform Manifold Approximation and Projection (UMAP) plots shows the distribution of different B cell population. (C) Distribution ratio of B cells among the Imiquimod (IMQ) and normal control (NC) groups.



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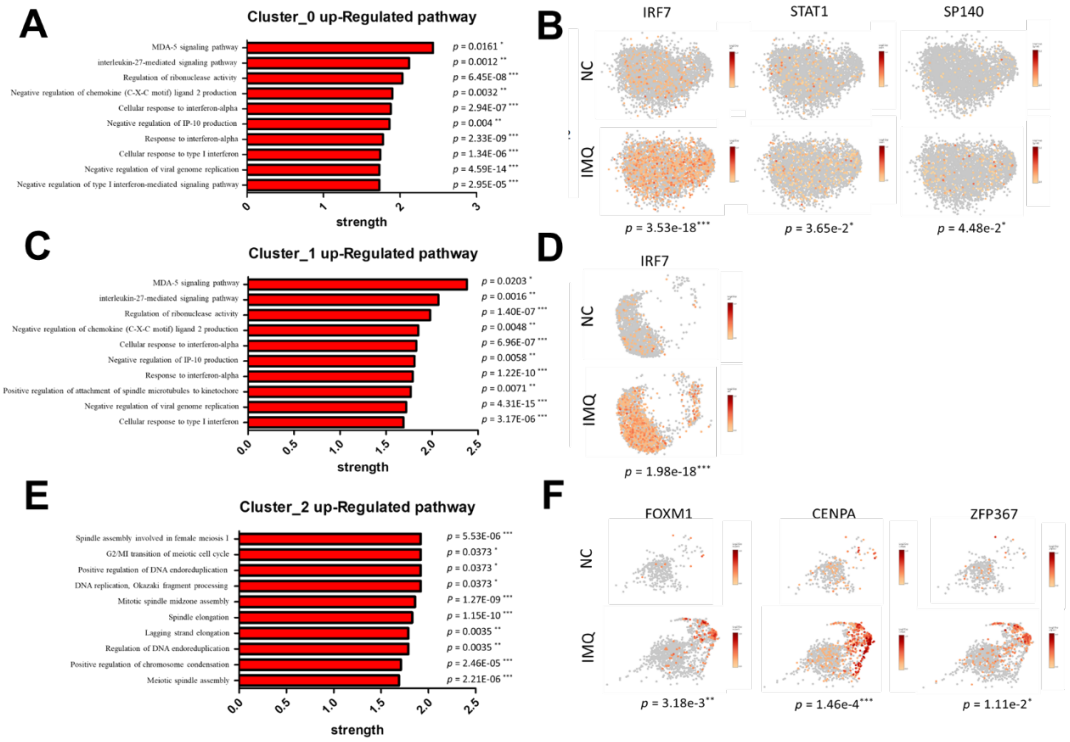

Fig. 2 Pathways and Transcriptional factors (TFs) analysis of the interested clusters and expression profiles of different TFs by comparing with IMQ and NC groups. The upregulating enriched GO terms (BP) using the thresholds gene fold change>1.5 and the gene expression profile of transcriptional factors that was identified using ChEA3 analysis in (A and B) cluster_1, (C and D) cluster_2 and (E and F) cluster_3.

Mechanisms of anti-dsDNA antibody production induced by commensal Streptococcus *mutans*: A study of molecular mimicry and NET formation in systemic lupus erythematosus

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共生鏈球菌 S. *mutans* 誘導的抗雙股 DNA 抗體產生機制：釐清共生鏈球菌於紅斑狼瘡之致病角色

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2. 國立臺灣大學醫學院臨床醫學研究所
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Abstract:

Background: SLE may be triggered by infections such as Enterococcus *gallinarum*, EBV, and SARS-CoV-2. We tested whether commensal streptococci can induce anti-dsDNA and elucidate the possible mechanism.

Methods: We tested whether S. *mutans*, can induce anti-dsDNA in a murine model. Antibodies(Ab) and MPO-DNA were quantified by ELISA or western blot.

Results: We examined whether commensal streptococci, like S. *mutans*, can induce anti-dsDNA in infective endocarditis (IE) patients(**Fig.1**A) and bacteremia models. In an *in vivo* mouse model, we observed anti-dsDNA production in mice with S. *mutans* bacteremia(**Fig.1**B). We identified that these Ab specifically recognized S. *mutans* surface protein glucosyltransferase I (GtfB,**Fig.2**A). Notably, recombinant GtfB alone induced anti-dsDNA antibody production in mice(**Fig.2**B), while a GtfB-deficient strain(NHS1ΔGtfB) reduced anti-dsDNA(**Fig.2**C). GtfB stimulates anti-dsDNA via molecular mimicry as shown by 3-D structural analysis (**Fig.2**D). We explored the correlation between NETosis biomarkers (MPO-DNA) and anti-GtfB and anti-dsDNA in SLE. It showed a weak positive correlation between MPO-DNA and anti-GtfB (r =0.27,p=0.26,n=20) and a weak negative correlation with anti-dsDNA (r=-0.1,p=0.69,n=20,**Fig.2**E). These findings suggest anti-GtfB from Streptococcus may be more related to NETosis.

Conclusion: Previous studies showed that Gtfs act as modulins to induce Th17-associated cytokines, contributing to neutrophil recruitment, valve damage, and vegetation formation in a IE model. In this study, we found anti-dsDNA was induced by GtfB through molecular mimicry. Another possible explanation is that S. *mutans* induces anti-dsDNA through NET formation, providing extracellular DNA as autoantigens(**Fig.2**F). Further studies are needed to clarify the roles of commensal streptococci in SLE pathogenesis.

A

	Male-to-female ratio	Age	Anti-dsDNA antibody-no.(%)	APL (Antiphospholipid antibodies) Anti-Cardiolipin IgG-no.(%)	Anti-Cardiolipin IgM-no.(%)	Anti-β2-Glycoprotein I IgG-no.(%)	APL no.(%)	APL + Anti-dsDNA antibody no.(%)
All deseminated systemic infection (n=50)	1.63	53.4 ± 20.7	13 (26)	22 (44)	23 (46)	10 (20)	27 (54)	30 (60)
IE (n=21)	2	52.4 ± 22.1	5 (23.8)	9 (42.1)	9 (42.8)	5 (23.8)	11 (52.3)	12 (57.1)
other deseminated systemic infection (n=29)	1.23	54.2 ± 19.9	8 (27.5)	13 (44.8)	14 (48.2)	5 (17.2)	16 (55.1)	18 (62)

B

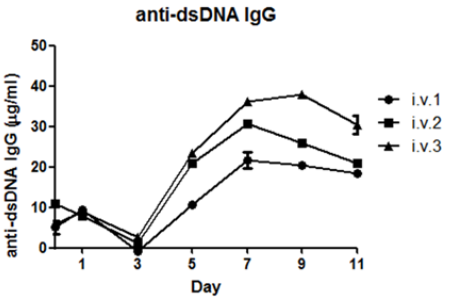

Fig1. Anti-dsDNA and pathogenic autoantibody production in IE and systemic infections from commensal streptococci, and in mice with *S. mutans* bacteremia A. Clinical studies found increased auto-dsDNA and anti-phospholipid antibodies in IE patients, and in those with systemic infections from commensal streptococci, suggesting these infections may induce autoantibody production. **B.** Mice infected intravenously with S. mutans GS5 strain (10^9 CFU) developed anti-dsDNA antibodies, detected by ELISA.

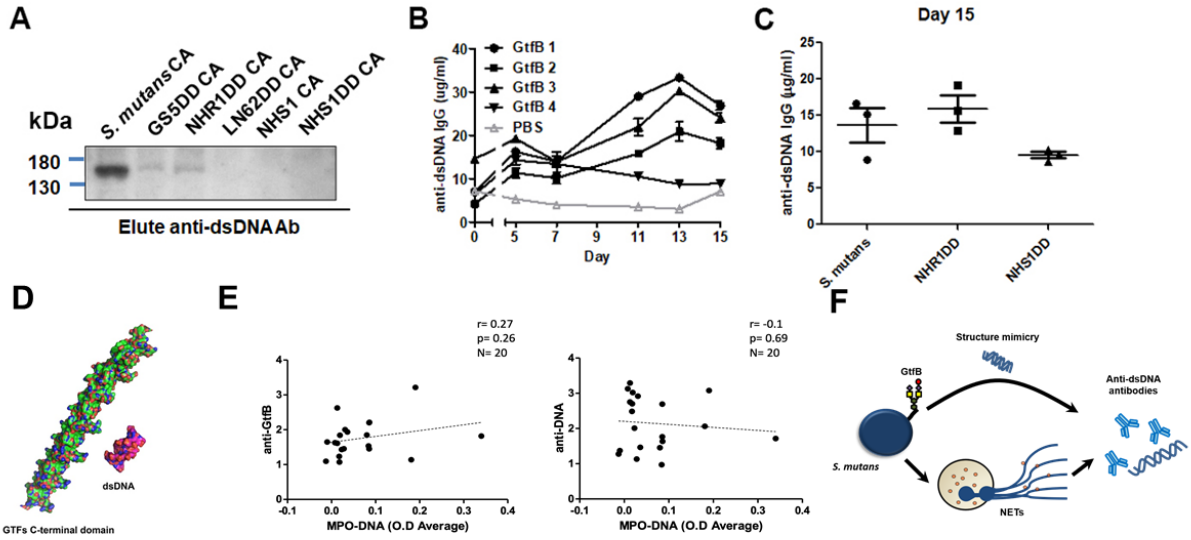

Fig2. GtfB's role in anti-dsDNA antibody induction via molecular mimicry and NET formation A. S. *mutans* has three GTF isoforms (GtfB, GtfC, GtfD) with 60-70% amino acid similarity. Anti-dsDNA from mice with S. *mutans* bacteremia were analyzed using Western blotting with bacterial cell wall associated proteins(CA) from wild type, GS5ΔGtfD(**GS5DD**), NHR1ΔGtfC/D (**NHR1DD**), LN62ΔGtfB/D(**LN62DD**), NHS1ΔGtfB/C(**NHS1**), and NHS1ΔGtfB/C/D strains(**NHS1DD**). The results indicated that anti-dsDNA antibodies recognized GtfB. **B.** Intravenous injection of recombinant GtfB in mice induced anti-dsDNA antibodies. **C.** Injection of the GtfB-deficient strain (NHS1ΔGtfB/C/D, strains(**NHS1DD**) reduced anti-dsDNA antibody production compared to the NHR1ΔGtfC/D strains(**NHR1DD**). **D.** The GtfB C-terminal domain may induce anti-dsDNA via molecular mimicry, as shown by crystallization and 3-D structural analysis. **E.** Anti-GtfB may be more closely associated with the NETosis biomarker MPO-DNA compared to anti-dsDNA. **F.** We propose two hypotheses for S. *mutans*-induced anti-dsDNA antibody production: 1) NETs contribute to autoantibody production, and 2) GtfB, acting as a DNA-mimic protein, stimulates anti-dsDNA production.