Study of Megakaryocytes in Systemic Lupus Erythematosus

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ABSTRACT

INTRODUCTION: Systemic Lupus Erythematosus (SLE) is an autoimmune disease associated with accumulation of autoantibodies and autoantigens in blood and organs. The formation of immune complexes (IC) plays a central role in SLE pathogenesis leading to organ damage and increased risks of cardiovascular disease and thrombosis. In SLE, platelets are activated by IC through activation of Fc receptor IIA (FcγRIIA), an immunoreceptor tyrosine-based activation motif. Mouse platelets lack an IC-recognizing receptor on their surface and in order to study the impact of IC in SLE, we thus developed a model of NZBxNZWF1 lupus-prone mice expressing the FcγRIIA transgene. The FcγRIIA expression led to accelerated nephritis and thrombosis and profound changes in platelet transcriptome, including an enriched IFN-regulated genes and dysregulated mitochondrial pathways. Mitochondria regulate numerous inflammatory pathways and dysregulation of mitochondria is implicated in the pathophysiology of SLE.

HYPOTHESIS: As platelets are anucleate, we hypothesize that changes observed in platelets during SLE reflect alterations that occurred in their mother cells, the megakaryocytes (MK). An SLE-prone environment could reprogram MK into a pro-inflammatory phenotype that plays a significant role in driving SLE pathology.

METHODS: NZBxNZWF1 lupus-prone mice expressing or not the FcγRIIA transgene were used. In vitro experiments were achieved using MK differentiated from progenitor cells, isolated from bone marrow through Lineage-negative selection. The effects of SLE-relevant stimuli were assessed on MK energy metabolism and functions. Spatial transcriptomic analyses were performed on bone marrow MK.

RESULTS: IC deposition was observed in organs from lupus-prone mice, noticeably in close contact with MK. Energy metabolism was altered in MK differentiated from SLE mice or in MK differentiated in an SLE-prone environment. Spatial transcriptomic analyses revealed different gene signature in MK from mouse with SLE, pointing to specific effects depending on their localization within the bone marrow.

CONCLUSION: An SLE-prone environment alters bioenergetic functions and gene expressions in bone marrow MK.

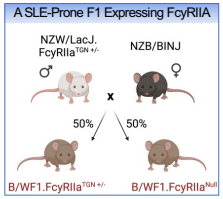
KEYWORDS: Lupus, Megakaryocytes, Metabolism, Inflammation, FcγRIIA, Immune-complexes

BACKGROUND

Systemic Lupus Erythematosus (SLE): Autoimmune disease characterized by a systemic inflammation. SLE is strongly sex-biased, affecting women nine times more frequently than men. In SLE, immune complexes (IC) formed by autoantibodies and autoantigens target multiple vital organs. There is no cure for SLE and patients can experience acute and cumulative organ damage and comorbidities, with increased risks of cardio-vascular disease and thrombosis.

FcγRIIA: Fc receptor containing an Immunoreceptor Tyrosine-based Activation Motif with an high avidity for IgG-IC. FcγRIIA is the sole FcγR expressed by platelets in humans and IC binding to FcγRIIA lead to platelet activation and aggregation.

Lupus-prone mouse model: A major obstacle in the study of platelet function in pathogeneses implicating antibodies is the absence of an IC-recognizing receptor on the surface of mouse platelets. We developed a model of NZBxNZWF1 lupus prone mice expressing the FcγRIIA transgene. This led to accelerated nephritis and thrombosis and profound changes in the platelet transcriptome with an enrichment for genes in the type-I IFN signaling pathway[1]. Sex bias is maintained in our model and female B/WF1. FcγRIIA^{TGN} mice have a shorter lifespan (29 weeks vs 37 weeks) compared with their B/WF1.FcγRIIA^{null} littermates. We further observed platelet activation, platelet secretion and release of mitochondria in this mouse model of SLE[2].



Megakaryocytes (MK): Large hematopoietic cells (~50–100 µm diameter) containing a multilobular, polyploid, nucleus (up to 128N). MK are producing circulating platelets. MK are typically considered as bone marrow (BM) cells and represent 0.05% of the cell population in the BM. However, platelet-producing MK also reside in the lung.

The mechanisms underlying initiation and progression of SLE are still not understood and we propose to investigate the role of Megakaryocytes in the disease.

An SLE-prone environment could modify MK leading to a significant role in driving SLE pathology.

RESULTS

1. IC interaction with MK in SLE

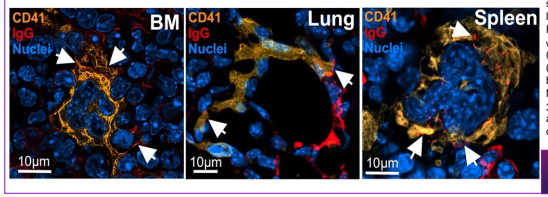


Figure 1 : Immunofluorescence staining of bone marrow (BM), lung and spleen in NZBxNZWF1 FcγRIIA^{TGN} SLE mice. Tissues were stained with an anti-CD41 (MK, orange), an anti-mouse IgG (Red) and a nuclear dye (Hoechst, blue). White arrows indicate IgG in MK. Images are representative of 3 different mice. All images were acquired using a Zeiss LSM 900 confocal microscope.

IC are found in MK from SLE mice

2. MK Mitochondrial Network

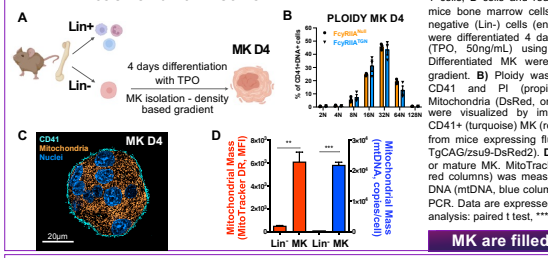


Figure 2 : **A)** Lineage positive (Lin+) cells (Granulocytes, T cells, B cells and red blood cells) were removed from mice bone marrow cells using magnetic beads. Lineage negative (Lin-) cells (enriched hematopoietic progenitors) were differentiated 4 days in presence of thrombopoietin (TPO, 50ng/mL) using Lineage negative cells (Lin-). Differentiated MK were isolated using a BSA density gradient. **B)** Ploidy was performed on MK at D4 using CD41 and PI (propidium iodide, DNA), n=3. **C)** Mitochondria (DsRed, orange) and nuclei (Hoechst, blue) were visualized by immuno-fluorescence in a mature CD41+ (turquoise) MK (representative of n=3) differentiated from mice expressing fluorescent mitochondria (B6D2F1-TgCAG/zsu9-DsRed2). **D)** Mitochondrial mass in Lin- cells or mature MK. MitoTracker Deep Red fluorescence (MFI, red columns) was measured by cytometry . Mitochondrial DNA (mtDNA, blue columns) was quantified by quantitative PCR. Data are expressed as mean ± SEM, n=5. Statistical analysis: paired t test, *** p<0.001,** p<0.01.

MK are filled with mitochondria

3. MK Metabolism in SLE

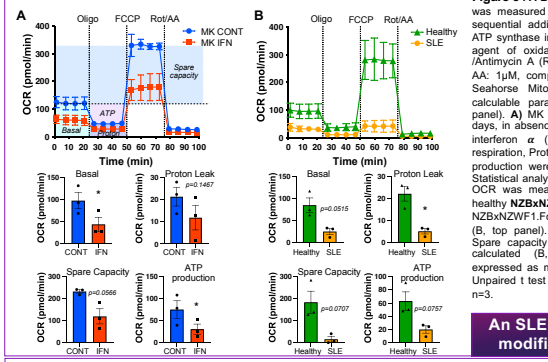


Figure 3 : **A-B)** Oxygen consumption rate (OCR) was measured in MK (5,000 cells) following a sequential addition of Oligomycin (Oligo: 4µM, ATP synthase inhibitor), FCCP (1µM, uncoupling agent of oxidative phosphorylation), Rotenone /Antimycin A (Rot: 1µM, complex I inhibitor and AA: 1µM, complex III inhibitor). Representative Seahorse Mito Stress Test assay indicating calculable parameters is illustrated in A (top panel). **A)** MK (**C57BL6J**) were differentiated 4 days, in absence (CONT, blue) or in presence of interferon α (IFN, 1,200 U/mL, red). Basal respiration, Proton Leak, Spare capacity and ATP production were calculated (A, bottom graphs). Statistical analysis: Paired t test,*p<0.05, n=3. **B)** OCR was measured in MK differentiated from healthy **NZBxNZWF1.FcγRIIA^{TGN}** mice (green) or NZBxNZWF1.FcγRIIA^{TGN} mice with SLE (orange) (B, top panel). Basal respiration, Proton Leak, Spare capacity and ATP production were also calculated (B, bottom graphs). Data are expressed as mean ± SEM. Statistical analysis: Unpaired t test with Welch's correction, *p<0.05, n=3.

An SLE-prone environment modifies MK metabolism

4.1 Approach Used for the Spatial Transcriptomic Profiling

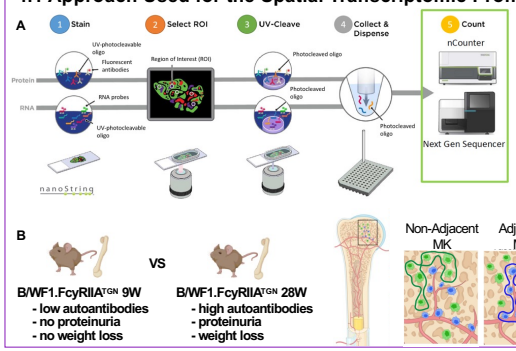


Figure 4 : **A)** GeoMx Digital Spatial Profiler workflow summary[3]. ROI = region of interest. **B)** Experimental design used in spatial transcriptomic. Femur from healthy B/WF1. FcγRIIA^{TGN} mouse (9 weeks old = 9W) and SLE B/WF1.FcγRIIA^{TGN} mouse (28 weeks old = 28W) were stained with endomucin (sinusoidal vessels) and CD41 (MK). ROI containing MK adjacent to the vasculature and MK non-adjacent to the vasculature were designed. 8 to 10 nucleated cells expressing CD41 were selected per ROI and 6 ROI per mouse were collected.

4.2 Spatial Transcriptome of MK subpopulations in SLE

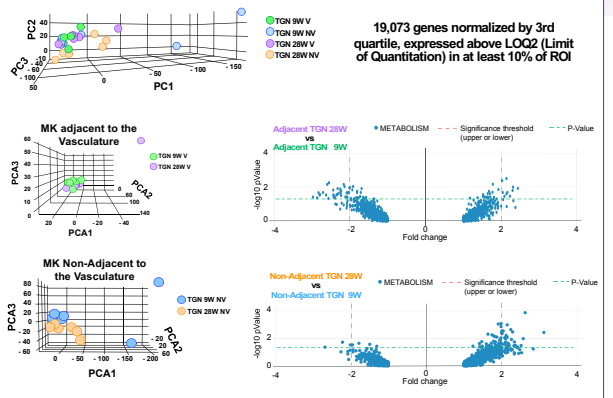


Figure 5 : Differential gene expression analysis. **A)** Principal component analysis (PCA) of the 19,073 mRNA sequenced by spacial transcriptomic in all sample, each dot represents one ROI (MK Adjacent to blood vessel = V; MK Non-Adjacent to blood vessel= NV). **B-C)** PCA (left panels, 19,073 genes) and Volcano Plots (right panels, around 1,900 gene of metabolism pathway) were generated. Significance threshold and P-value are represented. Data represent the difference between 6 MK-ROI from a B/WF1.FcγRIIA^{TGN} mouse with lupus (28 weeks old = 28W) and 6 MK-ROIs from a B/WF1.FcγRIIA^{TGN} mouse before any symptom of lupus (9 weeks old = 9W). **B)** Comparison of MK adjacent to the vasculature. **C)** Comparison of MK non-adjacent to the vasculature. Data analyses were made using Nanostring platform and normalized by 3rd quartile using DNA content.

MK have different gene signatures depending on their localization within the bone marrow during SLE.

CONCLUSION

An SLE-prone environment affects MK :

1. Immune complexes interact directly with MK in mice with SLE
2. MK metabolism is strongly decreased in an SLE-prone environment
3. Spatial transcriptomic analyses reveal MK heterogeneity in SLE with a tendency to more effects of SLE on MK non-adjacent to the vasculature.

Those observations suggest MK may contribute to progression of SLE disease through modification of their RNA content and functions.

This research could reveal MK as a new player in the progression of SLE. Additional investigations should be performed to evaluate how immune complexes directly impact MK functions and metabolism. MK could become a new therapeutic target in SLE.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Melki I, Allaeys I, Tessandier N, et al. FcgammaRIIA expression accelerates nephritis and increases platelet activation in systemic lupus erythematosus. *Blood*. 2020;136(25):2933-2945.

[2] Melki I, Allaeys I, Tessandier N, et al. Platelets release mitochondrial antigens in systemic lupus erythematosus. *Sci Transl Med*. 2021;13(581).

[3] *nanostring.com*

Illustrations were realized using Biorender

INFORMATION

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Disclosures: None