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Original Article

DNAJA3 regulates B cell development and immune function

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ABSTRACT

Background: DnaJ homolog subfamily A member 3 (DNAJA3), also known as the tumorous imaginal disc (Tid1), is shown to be crucial in T cell development. DNAJA3 functions as a tumor suppressor implicated in lymphocyte development and survival. However, the role of DNAJA3 in B cell development and immune function remains unknown. In this study, we utilized a mouse model of B cell-specific DNAJA3 knockout (CD19-Cre/$^{+}$; DNAJA3$^{flx/flx}$) to investigate the physiological function of DNAJA3 in B cell development and immune function.

Methods: We characterized B cell populations in various developmental stages and examined mitochondrial content and function between control and DNAJA3 KO using flow cytometry analysis. DNAJA3 and OXPHOS protein complexes in sorted B cells between mice groups were compared using immunoblot techniques. The activity of B cell blastogenesis in splenocytes was measured by performing CFSE and MTT assays. Furthermore, immunoglobulin production was detected using the ELISA method.

Results: DNAJA3 deficiency decreases from pro B cells to immature B cells. The overall B220^{+} population in the bone marrow and secondary immune organs also decreased. B cell subpopulations B1 (B1b) and B2 significantly decrease. The B cell blastogenesis activity and immunoglobulin production decreased in DNAJA3 KO mice. Mechanistically, DNAJA3 deficiency significantly increases dysfunctional mitochondria activity and decreases mitochondrial mass, membrane potential, and mitochondria respiratory complex proteins. These factors could have influenced B cell differentiation during development, differentiation to antibody-secreting cells, and immune activation.

Conclusion: Overall, our study provides supportive evidence for the role of DNAJA3 in B cell development and function.

Introduction

DNAJA3 (Tid1) is a mitochondrial co-chaperone with a conserved DnaJ protein domain to interact with the site for ATPase activation in heat shock protein 70 (HSP70). Physiologically, DNAJA3 is widely involved in protein folding, assembly, translocation, and degradation during cell growth and development [1–3]. Aside from HSP70, DNAJA3's client proteins are involved in intracellular signaling pathways related to cell survival, proliferation, and apoptosis [4–6]. Consequently, the interaction between DNAJA3 and its binding molecules can influence cellular functions. DNAJA3 was discovered as a tumor suppressor since the discovery of Tid56 in Drosophila from recessive-lethal tumor suppressor genes screening [7]. Subsequently, overexpression of DNAJA3 negatively regulated NF-κB to induce growth arrest and death in osteosarcoma cell lines (SAOS & U2OS) and suppressed tumor growth of human melanoma cells (A375SM) in nude mice [8,9]. The loss of DNAJA3 expression in basal carcinoma cells correlates with the loss of differentiation capacity of neoplastic cells, a common

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characteristic of malignant tumors similar to the *Drosophila* tumor model [10]. Further, DNAJA3's versatile function as a molecular chaperone extends its importance in early mammalian development and immune regulation. For example, homozygous DNAJA3 knockout mice die between 4.5 and 7.5 days of embryonic development [5]. Additionally, mice with tissue DNAJA3 gene deletion developed thymic atrophy, dilated cardiomyopathy, and promote muscle cell apoptosis during myogenesis [11–13]. In immune regulation, two studies have demonstrated that DNAJA3 regulated apoptotic resistance during activation-induced cell death of Th2 cells during T cell activation, while DNAJA3 deficiency reduced expression of the anti-apoptotic *bcl-2* gene [12,14].

Our body's defense mechanisms against infection are through either an innate or adaptive immune response. Among them, the B cell is an adaptive lymphocyte involved in antigen-specific recognition to eliminate infectious agents that escape an innate immune response. The activated B cells produce antibodies to remove extracellular pathogens and prevent them from spreading intracellularly. The development of B cells from bone marrow to different secondary immune organs, where it matures and exerts their function in eliminating pathogens alongside lasting protection from secondary infection, undergoes a tightly regulated chain of developmental stages [15]. Common lymphoid progenitors from hematopoietic stem cells become B lymphocyte progenitors that eventually diverge into two subsets, the B1 and B2 B cells, respectively. The B1 cells originate in the fetal liver, where they stay to mature and go to peritoneal cavities, then they further differentiate into B1a and B1b cell subsets. The B2 cells start development from bone marrow which further divides into follicular B (FO B) and marginal B cells (MZ B) that undergo further maturation in the spleen and other secondary immune organs. B1 and B2 cells have specialized functions based on varying phenotypes, localization, and activation [16]. The two B cell subpopulations elicit different immune responses, yet they function in overlapping activities within their respective B cell subsets. For instance, both the B1a and MZ B cells are analogous in their ability to mount an immediate response to blood-borne pathogens with their ability to produce natural antibodies. Meanwhile, the B1b and FO B cells contribute to an adaptive immune response by producing memory B cells and short- or long-lived plasma cells, respectively [17,18].

Development, survival, proliferation, differentiation, and subsequent activation of B cells require constant shifting of metabolic activity. Further, metabolic pathways, including oxidative phosphorylation and glycolysis, are important during B cells' development in the bone marrow and peripheral immune organs [19]. DNAJA3 functions as a mitochondrial co-chaperone protein and is involved in the homeostasis of its client proteins. Therefore, DNAJA3's functional versatility by interacting molecules localized in the cytosol or mitochondria would consequently influence the overall metabolic activity during the developmental stages and activation of B cells. In this study, we utilized a B cell-specific *DNAJA3*-deficient mouse model to evaluate the impact of DNAJA3 deficiency on B lymphocyte development in the bone marrow and secondary immune organs. Additionally, we sought to understand the impact of DNAJA3-deficiency during T-independent (TI) and T-dependent (TD) B cell activation and its implication on B cell immune response.

Materials and methods

Generation of B cell-specific DNAJA3-deficient mice and mouse genotyping

We generated a DNAJA3$^{flx/flx}$ mouse bearing a loxP-flanked DNAJA3 gene following the procedure described by Lo et al. [5]. We generated a B cell-specific *DNAJA3*-deficient mouse by using DNAJA3$^{flx/flx}$ mouse crossed with CD19-Cre$^{/+}$ transgenic mice. The CD19-Cre$^{/+}$ transgenic mice consist of cre insertion that interrupts the coding sequence of the *CD19* gene, resulting in CD19 deficiency in homozygotes. However, hemizygous mice for the cre insertion retain one functional CD19 allele that is phenotypically normal and ideal for B lineage-specific deletion of a floxed target gene like in the *loxP*-flanked DNAJA3 floxed locus shown in Supp. Fig. 1A [20,21]. Mice CD19-Cre$^{/+}$; DNAJA3$^{flx/flx}$ (DNAJA3 KO) contains cre recombinase gene that recognizes the loxP sequences at the ends of the DNAJA3 target gene in exon 1 to exon 2 of DNAJA3 locus resulting in DNAJA3 deletion in CD19 B lymphocytes (Supp. Fig. 1A). The sequence produces a frameshift mutation and thus, exhibits deficiency in DNAJA3 protein synthesis. Transgenic mice are genotyped using genomic DNA by PCR with IJKL (345bp), Cre (570bp), and DNAJA3 deletion locus (451bp) primers to differentiate DNAJA3$^{flx/flx}$ (Control) from B cell-specific DNAJA3-deficient mice (DNAJA3 KO) (Supp. Fig. 1B). Specimens for mice genotyping was taken from seven-day old pup tail or 4 weeks old mice blood. Mouse pups' tail (3 mm) was cut and collected in 50 mM NaOH solution, then heating at 100 °C for 1 h until the tail was completely homogenized. Afterwards, 1 M Tris–HCl (pH 8.0) was added and centrifuged. Afterwards, supernatant samples were collected and stored in −20 °C until use. The genotype screening was confirmed by PCR analysis. Detection of DNAJA3 KO mice utilized Cre primer (F:5′-CCGGTCGATGCAACGAGTGAT-3′, R:5′ACCAGAGTCATCCTTAGCGCC-3′) and deletion locus primer-EfloxpKL(F:5′-TGAGCCCACAAGTTTGAGACTAGC-3,R:5′ TAGCCCTTTAGCATCCTGGTGC-3′). while control mice were genotyped using IJKL primer (F:5′-GTTTAAGGCCAGTTTGTCTCAAAAC-3′, R:5′-ACTTGACTAGCCCTTTAGCATC-3′).PCR product was electrophoresed using 2–2.5% agarose gel for 30 min at 100 V and imaged (UVC1200-312, Major Science, USA).

Tissue collection and cell isolation

We performed daily records of the mice's weight after birth. All mice were euthanized at 10–12 weeks old, and spleen, thymus, bone marrow, mesenteric lymph node (MLN), and intestine Peyer's patches (PP) were harvested for appearance, tissue weight, B cell subset distribution, and functional analysis. Peritoneal exudate cells (PEC) are collected by lavage using 6 ml of 2% FBS-HBSS buffer. Spleen and thymus ground to single-cell suspensions with RPMI-1640 culture media containing 10% FBS. Mouse femurs and tibias cut at both ends were flushed with D-PBS supplemented with 2% FBS to collect bone marrow cells. Blood (0.5–1 ml) was collected through submandibular bleed and cardiac puncture into heparinized tubes. All suspended cells were stained with EtBr/AO and counted under a blue fluorescence microscope. For the collection of T cells and B cells to confirm the DNAJA3 gene is B cell-specifically knock-down and for mitochondrial analysis, CD3$^+$ and CD19$^+$ cells from splenocytes were sorted using Fluorescence Activated Cell Sorter (BD FACSAria IIu, USA). Protein extracts from sorted cells or splenocytes were prepared using RIPA buffer, and the cellular DNAJA3 protein expression was examined by immunoblotting. The purity (~98%) of CD3$^+$ T cells or CD19$^+$ B cell was verified by flow cytometry analysis.

Flow cytometry

Flow cytometric analysis of BM cells, whole blood (50 μl), thymus, MLN, PP, and splenocytes was performed with the following monoclonal antibodies conjugated to phycoerythrin (PE), fluorescein (FITC), Brilliant Violet 421, Cyanine5, or PE-Cy5. Cells (2–5 × 10^5) were stained with antibodies enumerated below for 30 min at 4 °C in the dark, washed twice with FACS buffer, and suspended in 1 ml FACS buffer before flow cytometry analysis. Bone marrow, spleen, blood, MLN, PEC, and PP cells (2 × 10^5) were washed with FACS buffer and stained with anti-mouse B220-PE antibody to check overall B220$^+$ cells. PEC cells (2 × 10^5) were stained with anti-mouse CD19-PE Cyanine5 (Biolegend, USA, 115,509) and anti-mouse CD43-PE (eBioscience, USA, 12-0431-82) antibodies to check B1 and B2 cell populations. Meanwhile, anti-mouse CD5-FITC (Biolegend, USA, 100,605) and anti-mouse IgM-Brilliant Violet 421 (Biolegend, USA, 406,532) antibodies were used to check B1 subpopulation, B1a, and B1b. Bone marrow cells were stained

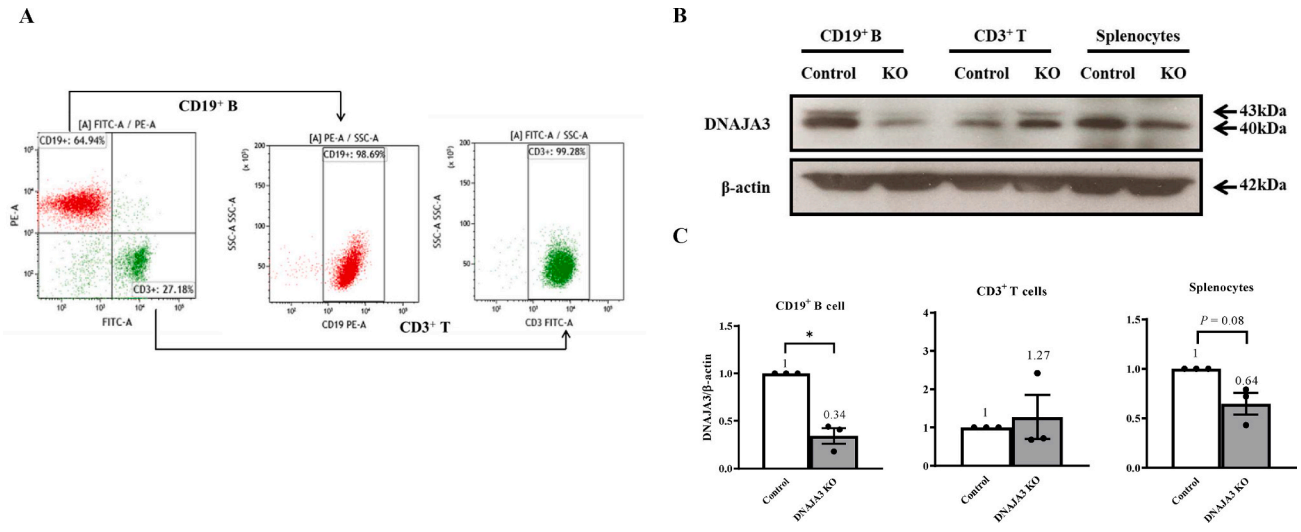

Fig. 1. A–C. Deficient DNAJA3 protein expression in DNAJA3 KO mice. $CD19^+$ B cells and $CD3^+$ T cells sorted from control and DNAJA3 KO mice splenocytes were collected for protein extraction, and their purity was determined by flow cytometry and showed in the histogram **(A)**. The expression level of short-form and long-form DNAJA3 proteins was measured by Western Blot with a specific antibody **(B)**. The densitometry of DNAJA3 protein expression in the bands on the blot was quantitatively analyzed by image J software. Values are presented as mean of the density ratio of DNAJA3 protein expression in relation to β-actin of control and DNAJA3 KO mice **(C)**. Confirmed low expression of DNAJA3 protein in $CD19^+$ B cells and whole splenocytes of DNAJA3 KO mice. The symbol * as $p \leq 0.05$ in comparison to control mice.

with anti-mouse CD43-PE, anti-mouse CD19-PE Cyanine5, and anti-mouse IgM-Brilliant Violet 421 antibodies and gated to check Pre-pro B, Pro B, Pre-B, and immature B stages. Blood, spleen, MLN, and PP cells were also stained with anti-mouse CD43-PE, CD19-PE Cyanine5, anti-mouse IgM-Brilliant Violet 421, IgM PE-Cyanine 5, and anti-mouse IgD-FITC antibodies to verify immature and mature B cells. To identify Follicular B ($B220^+CD23^+CD21^+IgM^+$) and Marginal B ($B220^+CD23^-CD21^+IgM^+$) cell populations in splenocytes cells stained with antibodies (Biolegend, USA) and gated. Expression of lymphocyte antigen activation marker in $B220^+$ cells was determined using CD25-PE (Biolegend, USA, 101,903) and CD69-FITC (Biolegend, USA, 104,505) antibodies. Expression of co-stimulatory molecules in $B220^+$ cells was performed by staining splenocytes with CD40-FITC (BD, USA, 553,723), CD80-PE (Biolegend, USA, 104,707), and CD86-FITC (Biolegend, USA, 105,005). MHC I and MHC II antigen-presentation surface markers were also determined using H-$2K^b$-PE (eBioscience, USA, 4,344,445) and I-A^b-FITC (Biolegend, USA, 116,405), respectively. Activated B cell populations after antigen stimulation were stained with anti-mouse/human GL7-FITC (Biolegend, USA, 144,603) for germinal center B cell marker. Plasmablast and plasma cell populations were stained with anti-mouse CD138-PE (Biolegend, USA) and TACI/CD267 (eBioscience, USA, 17-5942-81) antibodies. Thymocytes were stained with markers anti-mouse CD3e-PE Cyanine5, anti-mouse CD3e-PE Cyanine5, and anti-mouse CD8a-PE to verify T cell populations in Control and DNAJA3 KO mice. Flow cytometry analysis and results were performed using Cytoflex S (Beckman Coulter, USA) and FlowJo™ v10.6 Software (BD Life Sciences). The average number of cells in representative samples of PEC, blood, spleen, thymus, MLN, PP, or BM under flow cytometry was used to calculate the absolute number of positive cells.

T-distributed stochastic neighbor embedding (t-SNE) analysis

The t-SNE analysis used FlowJo following the learning configuration opt-SNE (iterations = 1000, perplexity = 30). The learning rate used was based on the opt-SNE approach. Each flow cytometry sample file was concatenated into a single file (Control or DNAJA3 KO), and distinct populations were defined and used to perform t-SNE analysis.

B cell blastogenesis assay

Splenocytes were collected from 10 to 12 weeks old mice. RBC lysed splenocytes (1×10^6) were cultured in 96 well plates treated with or without LPS 5 μg/ml (TI antigen) or a combination of α-IgM and α-CD40 5 μg/ml (TD antigen) for 48 h in RPMI-1640 culture media at 37 °C in 5% CO_2 incubator. B cell proliferation was measured by MTT and CFSE assays. For the MTT assay, stimulated splenocytes were added with 15 μl/well of MTT reagent (5 mg/ml) and incubated for 40 min at 37 °C in the dark. After 40 min, 100 μl/well of 0.06 N acid-isopropanol was added, and absorbance of OD_{570nm} was measured using a spectrophotometer (SPECTRA max 340 PC, Tekon Technologies, USA). For the CFSE assay, splenocytes (5×10^7) in pre-warmed RPMI-1640 culture media were labeled with 5 μM CFSE dye for 10 min at 37 °C in a 5% CO_2 incubator. After washing, CFSE-labeled splenocytes (1×10^6 cells/well) were seeded and then stimulated with LPS (5 or 10 μg/ml) and incubated for 72 h at 37 °C. Activated cells by antigen stimulation were collected, and cells were stained with anti-mouse B220 antibodies for flow cytometry analysis.

ELISA assay for IgG and IgM antibody production

The supernatant of cultured splenocytes stimulated with LPS was collected, and the production of IgG and IgM antibody released was quantified using enzyme-linked immunosorbent assay following the kit protocol (eBioscience, USA, 88–50400 and 88–50470). In brief, the 96-well microplate was coated with diluted capture antibody for 12 h s at 4 °C, washed with washing buffer before adding blocking buffer for 2 h s, and washed twice. Diluted culture supernatants or standards were added and incubated for 2 h s before adding HRP-conjugated anti-mouse secondary antibody for 1hr and washed. Afterward, substrate solution was added until blue coloration was detected, then stop solution was added, and OD_{450nm} was measured with a spectrophotometer. IgG and IgM concentrations were computed using the standard curve.

Immunoblotting

Splenocytes from control and DNAJA3 KO mice were collected and prepared for a single-cell suspension. Then, $CD3^+$ and $CD19^+$ cell populations were sorted using FACS. Protein extracts from sorted cells or

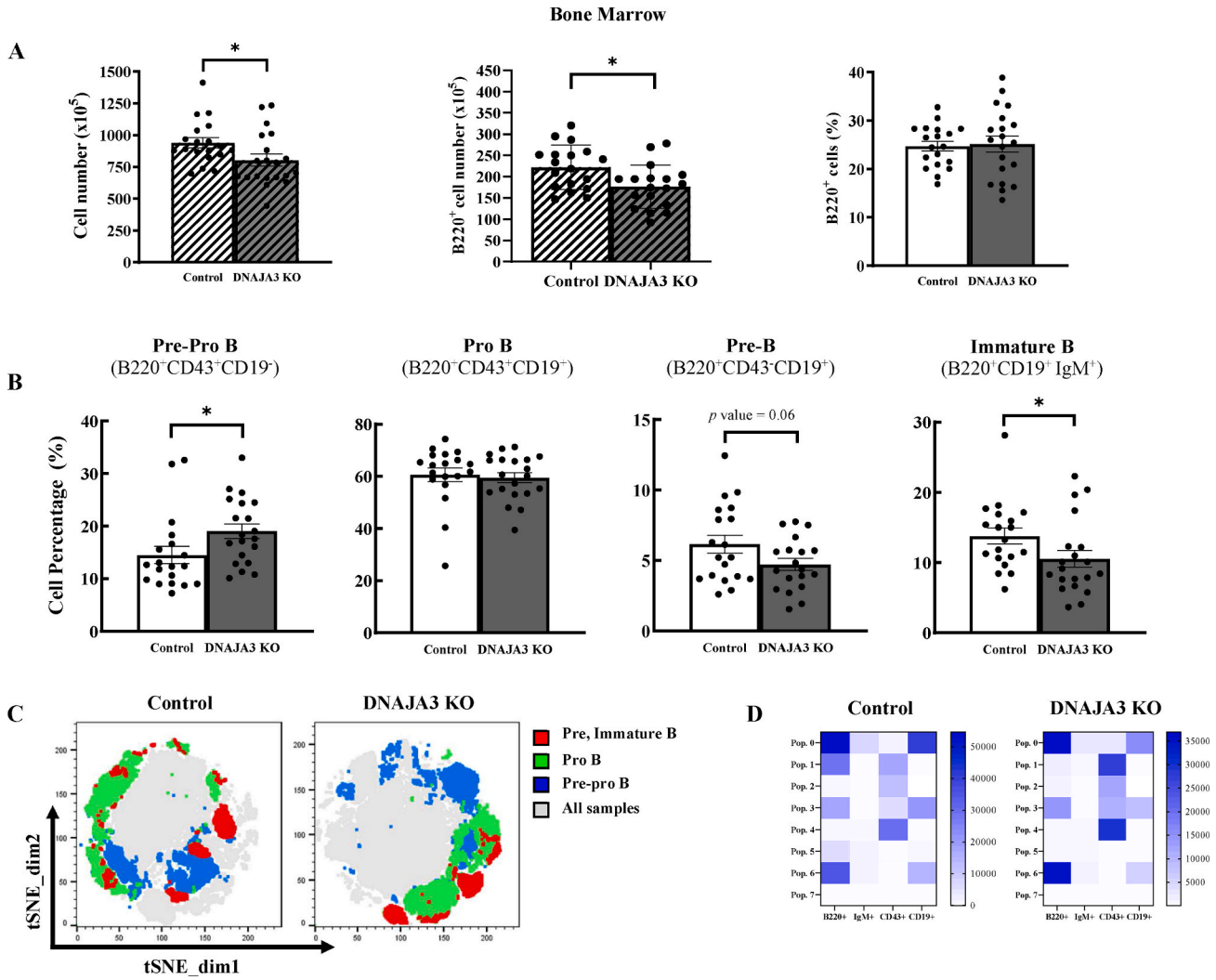

Fig. 2. A–D. DNAJA3 deficiency disrupted early stages of B lymphocyte development in bone marrow. Total bone marrow cells, $B220^+$ cell number, $B220^+$ cell percentage, pre-pro-B ($B220^+CD43^+CD19^-$), pro-B ($B220^+CD43^+CD19^+$), pre-B ($B220^+CD43^-CD19^+$), and immature B ($B220^+CD19^+IgM^+$) cells were measured and quantified by flow cytometry with specific antibodies. $B220^+$ cell percentage showed no significant reduction, but total bone marrow cells and $B220^+$ cell numbers significantly decreased between control and DNAJA3 KO mice **(A)**. Flow cytometry analysis of early B cell stages in bone marrow showed an increased percentage of Pre-pro B cells. In contrast, a significant reduction of Pre-B and Immature B cells in DNAJA3 KO mice ($N = 20$) occurred in comparison to Control ($N = 19$) **(B)**. Concatenated flow cytometry files from manually gated flow samples for Control and DNAJA3 KO were used for T-distributed stochastic neighbor embedding (tSNE) plot analysis. Overlay of Pre-pro B, Pro B and Pre, Immature B cell populations tSNE plot for analysis of distribution of different B cell stages between Control and DNAJA3 KO **(C)**. Heatmap analysis of population clusters from tSNE plots versus counts of surface markers $B220^+$, IgM^+, $CD43^+$ and $CD19^+$ between Control and DNAJA3 KO mice **(D)**. Values are expressed as mean ± SE. The symbol * as $p \leq 0.05$ in comparison to control mice.

splenocytes were prepared using a mixture of protease inhibitors and RIPA lysis buffer. The extracted protein mixed with 2× sample buffer was processed with heating treatment and then electrophoresed in 10% SDS-PAGE gel at 70 V for 10 min, followed by 100 V for 100 min. Protein bands were blotted into a PVDF membrane (GE Healthcare Life Science, 10,600,023, Germany) and electrophoresed at 100 V for 60 min. Washed membranes were soaked in blocking buffer (5 w/v % skim milk in TBST) for 2 h and washed twice with TBST. Primary antibodies included anti-mouse Tid1 (Thermo Fisher, MA5-12267, USA), anti-mouse β-actin (Abcam, ab6276, USA), and total OXPHOS (Abcam, ab110413, USA) were used and incubated at 4 °C overnight. After washing, rabbit anti-mouse IgG-HRP (Sigma, A9044, USA) as a secondary antibody was added and incubated for 1 h at room temperature. Afterward, the membrane was washed with TBST twice before adding an enzyme chemiluminescent (ECL) reagent in the dark. Immunoblot was developed in chemiluminescence film (GE Healthcare Life Science, Japan, 28,906,838). Protein bands were analyzed using ImageJ software [22].

Mitochondrial assay of activated B cells

Assay for evaluating mitochondria function, mass, and membrane potential are followed from protocols described in Monteiro et al. paper (2020). In brief, cells are stained with $B220^+$ antibody, Mitotracker Red (PE), and Green (FITC) (Invitrogen, USA, M7514, M7512) to analyze mitochondrial functionality. The Mitochondria mass of $B220^+$ cells was identified using $B220^+$ and Mitotracker Green, while mitochondria membrane potential was detected using JC-1 Dye (Invitrogen, USA, T3168) following manufacturing protocol. All assays were analyzed by flow cytometry analysis, and results were performed using Cytoflex S (Beckman Coulter, USA) and FlowJo™ v10.6 Software (BD Life Sciences).

Statistics

All data are presented as means ± S.E.M. the statistical difference between Control and DNAJA3 KO group was assessed with unpaired

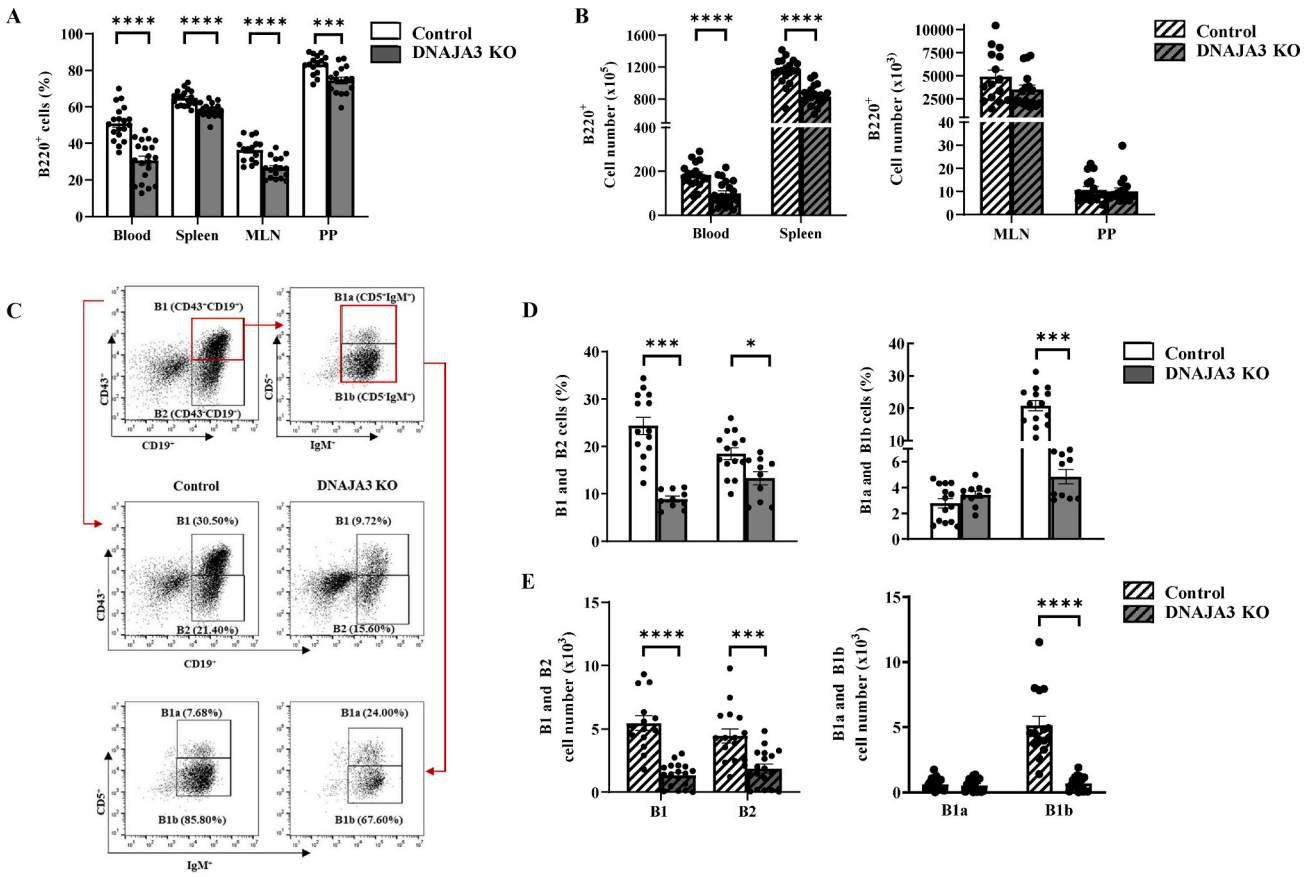

Fig. 3. A–E. B cell-specific DNAJA3 knock-out reduced total B220$^+$ B cells population in secondary immune organs and two B subsets in PEC, respectively. Single-cell suspension of secondary immune organs such as blood, spleen, mesenteric lymph node (MLN) and Peyer's patch (PP) were collected, then B220$^+$ cell percentage was determined by flow cytometry. Analysis results showed significant reduction of B220$^+$ B cell population percentage in secondary immune organs of DNAJA3 KO mice compared to Control **(A)**. Actual total B220$^+$ cell numbers in blood and spleen significantly decreased but not MLN and PP between control and DNAJA3 KO mice **(B)**. B cell progenitors are diverged into B1 and B2 B cell population. The majority of B1 progenitors will migrate to peritoneal cavity where it will mature and further differentiate into B1a and B1b subset. Gating strategy for B1 (CD43$^+$CD19$^+$) and B2 (CD43$^-$CD19$^+$) populations and sub-populations B1a (CD5$^+$IgM$^+$) and B1b (CD5$^-$IgM$^+$) of B1 B cells **(C)**. Bar graphs shows significant reduction of B1 and B2 cell percentage in DNAJA3 KO mice compared to control. Between B1a and B1b subsets, there is a major reduction in B1b cell percentage in DNAJA3 KO mice compared to control **(D)**. Similar patterns of reduction of B1, B2 and B1b cell numbers are shown **(E).** Values are expressed as mean ± SE. The symbol * as $p \leq 0.05$, *** as $p \leq 0.001$ and **** as $p \leq 0.0001$ in comparison to control mice.

Student T-test with a significance level of p value ≤ 0.05. All statistical analysis was performed using GraphPad Prism version 8.0 (GraphPad Software, La Jolla, California, USA).

Results

DNAJA3 KO mice showed abnormal spleen, reduced cell number population, and DNAJA3 protein expression deficiency in CD19$^+$ B cells

Body weight, primary (bone marrow and thymus), and secondary immune organs (spleen, blood, MLN, PP) were weighed, and total cells were counted. There was no significant difference between control and DNAJA3 KO mice in body weight, thymus weight index, or MLN weight index (Supplemental Table 1). However, spleen weight index and bone weight index were significantly decreased in DNAJA3 KO mice (Supplemental Table 1). The total cell numbers of primary and secondary immune organs showed a definite decrease in bone marrow, blood, and spleen in DNAJA3 KO mice but no difference in the thymus (Supplemental Table 2 and Table 3). Total thymus cell number and T cell subpopulations (CD3$^+$, CD4$^+$, CD8$^+$) did not differ between control and DNAJA3 KO mice (Supplemental Table 3). Complete blood count analysis showed a noticeable decrease of lymphocytes in DNAJA3 KO compared to control mice, while red blood cells, platelets, hematocrit, and hemoglobin between mice groups showed no difference (Supplemental Table 4, $p = 0.015$). We verified the protein expression level of the two DNAJA3 protein regions, long-form DNAJA3$_L$ (43-kDa) and short-form DNAJA3$_S$ (40-kDa) in sorted CD19$^+$ B cells, CD3$^+$ T cells, and total splenocytes (Fig. 1A–B). The DNAJA3 protein expression in sorted CD19$^+$ cells significantly decreased in DNAJA3 KO mice compared to control by 66% (Fig. 1C). The decreased DNAJA3 protein expression in CD19$^+$ B cells confirms DNAJA3 KO mice are deficient in DNAJA3 protein, specifically in B lymphocytes.

Attenuated early B cell development in DNAJA3 KO mice

To know the impact of DNAJA3 deficiency on B lymphocyte development, we first examined the effectof DNAJA3 deficiency on early B cell development in the bone marrow. Total bone marrow cell number was significantly decreased in DNAJA3 KO mice (Fig. 2A; $p = 0.03$). Similarly, the marrow B220$^+$ cell number was reduced ($p = 0.011$). However, the B220$^+$ cell percentage did not differ between mice groups (Fig. 2A; $p = 0.8184$). Four surface markers were used to distinguish Pre-pro B (B220$^+$CD43$^+$CD19$^-$IgM$^-$), Pro-B (B220$^+$CD43$^+$CD19$^+$IgM$^-$), Pre-B (B220$^+$CD43$^-$CD19$^+$IgM$^+$), and Immature B (B220$^+$CD43$^-$CD19$^+$IgM$^+$) cell population in bone marrow (Fig. 2B). Results show that B cell number in the Pre-pro B stage was significantly

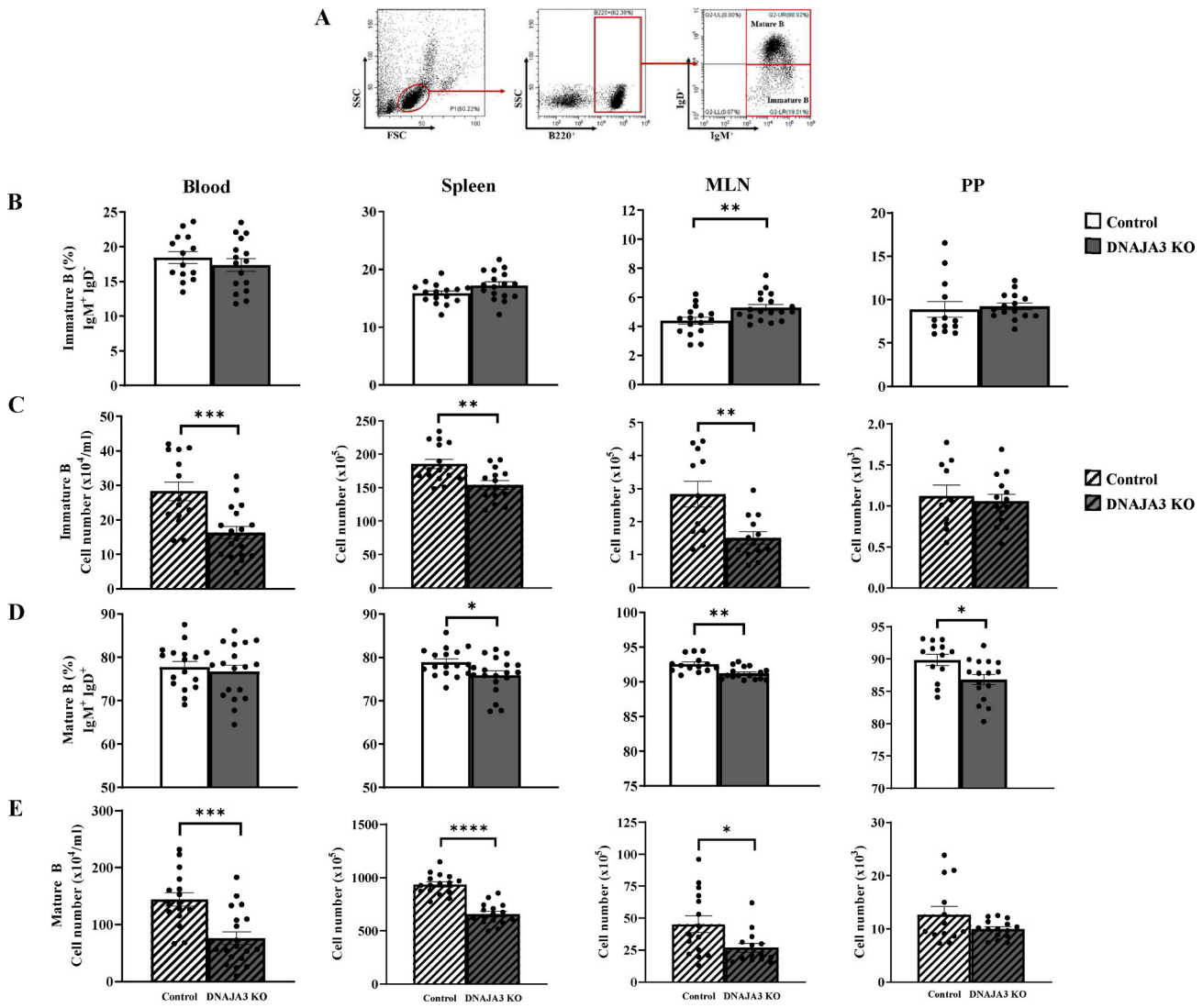

Fig. 4. A–E. Decreased immature and mature B cells in secondary immune organs of DNAJA3 KO mice. Gating strategy in late B cell development stage, immature and mature B cells were stained with IgM^+ and IgD^+ marker in $B220^+$ cells for flow cytometry analysis **(A).** Cell percentage of immature B cells (IgM^+IgD^-) of DNAJA3 KO mice did not significantly differ in blood, spleen, and PP except for MLN **(B)** but cell numbers decreased in blood, spleen, and MLN in DNAJA3 KO mice **(C).** Mature B cell (IgM^+IgD^+) percentage showed slight reduction in blood but significantly in spleen, MLN and PP **(D).** Meanwhile, cell numbers showed dramatic decrease of mature B cells in blood, spleen and MLN but not in PP **(E).** Values are expressed as mean ± SE. The symbol * as $p \leq 0.05$, ** as $p \leq 0.01$, *** as $p \leq 0.001$ and **** as $p \leq 0.0001$ in comparison to control mice.

increased in DNAJA3 KO mice ($p \leq 0.05$) along with a gradual reduction of B cell population from Pro-B stage towards Pre-B cell and then leads to a significant reduction in Immature B cells in DNAJA3 KO mice (Fig. 2B, $p \leq 0.04$). Visualization of B cell populations using tSNE analysis in bone marrow demonstrates that DNAJA3 KO mice have a less scattered distribution of Pre and Immature B cells compared to control mice (Fig. 2C). A consistent result based on a heatmap comparison of surface markers showed that DNAJA3 KO mice exhibit a higher count of $CD43^+$ cells, a marker in early B cells stages (Pre-pro B and Pro B) meanwhile control mice had higher counts of $B220^+$, $CD19^+$ and IgM^+ cells that are markers for Pre to Immature B cells (Fig. 2D). This shows that DNAJA3 deficiency in B cells will disturb the early B cell development in the bone marrow and may attenuate development towards B cell maturation.

Impaired sub-B cell lineage development and late B cell development in DNAJA3 KO mice

Two B-lineage progenitors develop into B-1 and B-2 populations after exiting the fetal liver and bone marrow [16]. The B-1 progenitors enter the peritoneal cavity turning into B-1 cells, and then peritoneal B-1 cells are further subdivided into the B-1a and B-1b. The B-2 progenitors enter the secondary lymph organs and differentiate into conventional B (B-2) cells. To determine the effect of DNAJA3 deficiency on later B lymphocyte development, we first investigated the $B220^+$ cell population in secondary immune organs. Considerably, DNAJA3 KO mice significantly decreased $B220^+$ cell population percentage in blood, spleen, MLN, and PP (Fig. 3A). In contrast, total $B220^+$ cell numbers only showed a significant reduction in blood and spleen but not in MLN or PP (Fig. 3B). To further examine total B1 ($CD43^+CD19^+$) and B2 ($CD43^-CD19^+$) as well as B1 subsets, B1a ($CD5^+IgM^+$) and B1b ($CD5^-IgM^+$) cells were detected in the peritoneal exudate cavity (PEC) of control and DNAJA3 KO mice (Fig. 3C). B1, B1b, and B2 cell population percentage and cell numbers in DNAJA3 KO mice dramatically decreased compared to the control. In contrast, B1a cell percentage or cell numbers did not differ between mice groups (Fig. 3D–E). Results suggest that DNAJA3 deficiency strongly affects lymphocyte progenitors B1 and B2 B cell subsets that are important in B cell differentiation B cells. Immature B cells respond to antigens to become mature B cells in

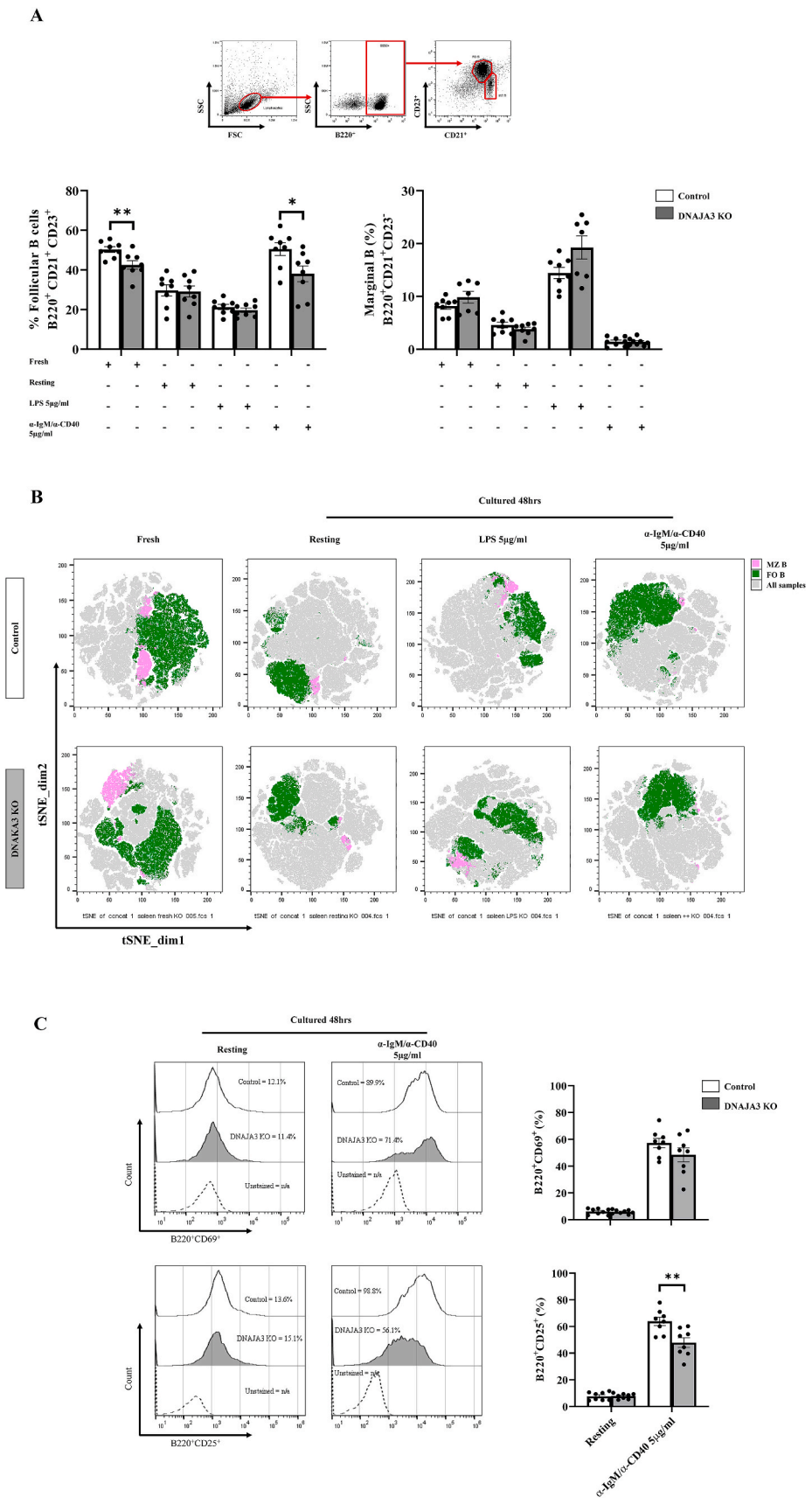

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Fig. 5. A–C. DNAJA3 deficiency decreased Follicular B cells and reduced the later activation marker expression of B cells. Splenocyte cells were collected from 12 weeks old mice. Cells (1×10^6) were cultured in 96 well plate treated with or without LPS 5 μg/ml (TI-1 antigen) or a combination of α-IgM and α-CD40 5 μg/ml (TD antigen) for 48 h. Follicular B (FO B) and Marginal B (MZ B) in flow cytometric analysis are characterized by $CD23^+CD21^+$ and $CD23^-CD21^+$ antibody markers, respectively. As anticipated FO B cells are induced with TD antigen. DNAJA3 KO mice showed significant decrease of FO B cells (%) versus control. In contrast, MZ B cells (%) were slightly high in DNAJA3 KO mice in T1 antigen stimulation **(A)**. Overlay of FO and MZ B populations tSNE plot between Control and DNAJA3 KO in different stimulation conditions **(B)**. $B220^+$ cells expressing early activated marker $CD69^+$ slightly decreased in cell cultured with TD antigen. Similarly, $B220^+$ expressing later activated marker $CD25^+$ significantly decreased in TD antigen stimulation in DNAJA3 KO mice compared to control **(C)**. The symbol * as $p \leq 0.05$ and ** as $p < 0.01$ in comparison to control mice.

secondary immune organs after exiting from the bone marrow. To examine the impact of DNAJA3 deficiency on the later stage of B cell development, cells collected from secondary immune organs, including blood, spleen, MLN, and PP, were stained with antibody surface markers to distinguish immature ($B220^+IgM^+IgD^-$) and mature B cells ($B220^+IgM^+IgD^+$). In DNAJA3 KO mice, cell percentages of immature and mature B cells in blood slightly decreased (1–5%) compared to control (Fig. 4B and D) but not as significant as cell numbers of immature B cells that reduced by 42.37% compared to control ($p = 0.0006$) similarly in mature B cells, a significant reduction of 47.29% was observed ($p = 0.0002$) (Fig. 4C and E). In spleen and MLN, the percentage of mature B cells, as well as the number of immature and mature B cells, were significantly decreased in DNAJA3 KO mice (Fig. 4C; $p = 0.003$ for spleen; $p = 0.004$ for MLN of immature B, 4 E; $p \leq 0.0001$ for spleen; $p = 0.021$ for MLN of mature B), except no difference in cell percentages of spleen immature B cells. In contrast, there is no difference in the number of immature and mature B cells in PP, while the cell percentage of mature B cells decreased by 3.36% (Fig. 4D; $p = 0.016$). Results suggest DNAJA3 KO mice showed consistent attenuation in immature B cells in the bone marrow and mature B cells in secondary immune organs.

Reduced FO B cell development and attenuated T-dependent B cell activation in DNAJA3 KO mice

The majority of mature B-2 cells reside within the marginal zone (MZ B cells) and lymphoid follicles (FO B cells) of the spleen and lymph nodes, where they encounter and respond to foreign antigens bound on follicular dendritic cells (DCs), activation, proliferate, and either differentiate into plasma cells or enter germinal center (GC) reactions [15,16,18]. Therefore, we investigated the FO B ($B220^+CD21^+CD23^+$) and MZ B ($B220^+CD21^+CD23^-$) cell population in the spleen with and without TI (LPS 5 μg/ml) or TD (α-IgM/α-CD40 5 μg/ml) antigen stimulation. DNAJA3 deficient mice showed significantly low FO B cells in

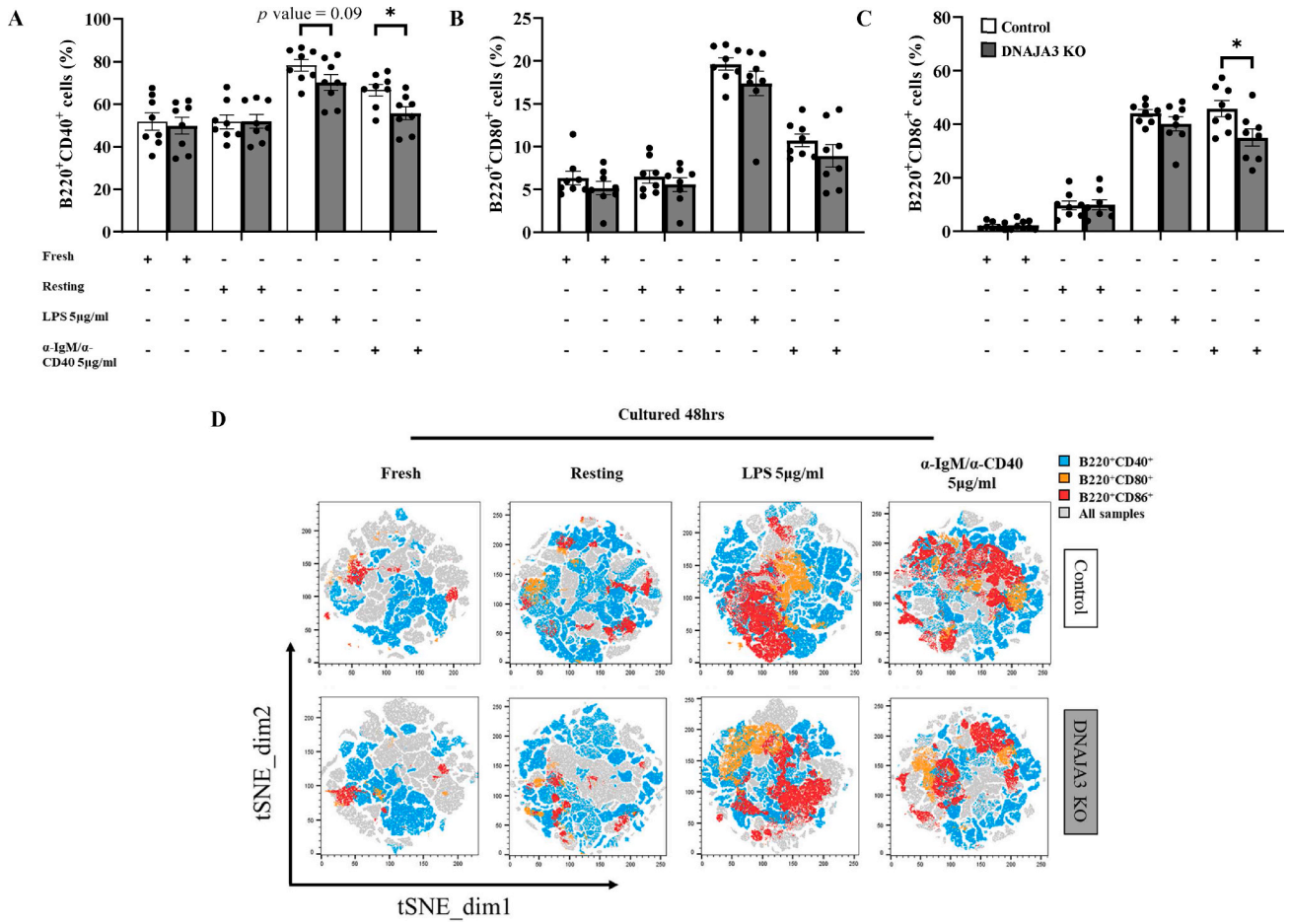
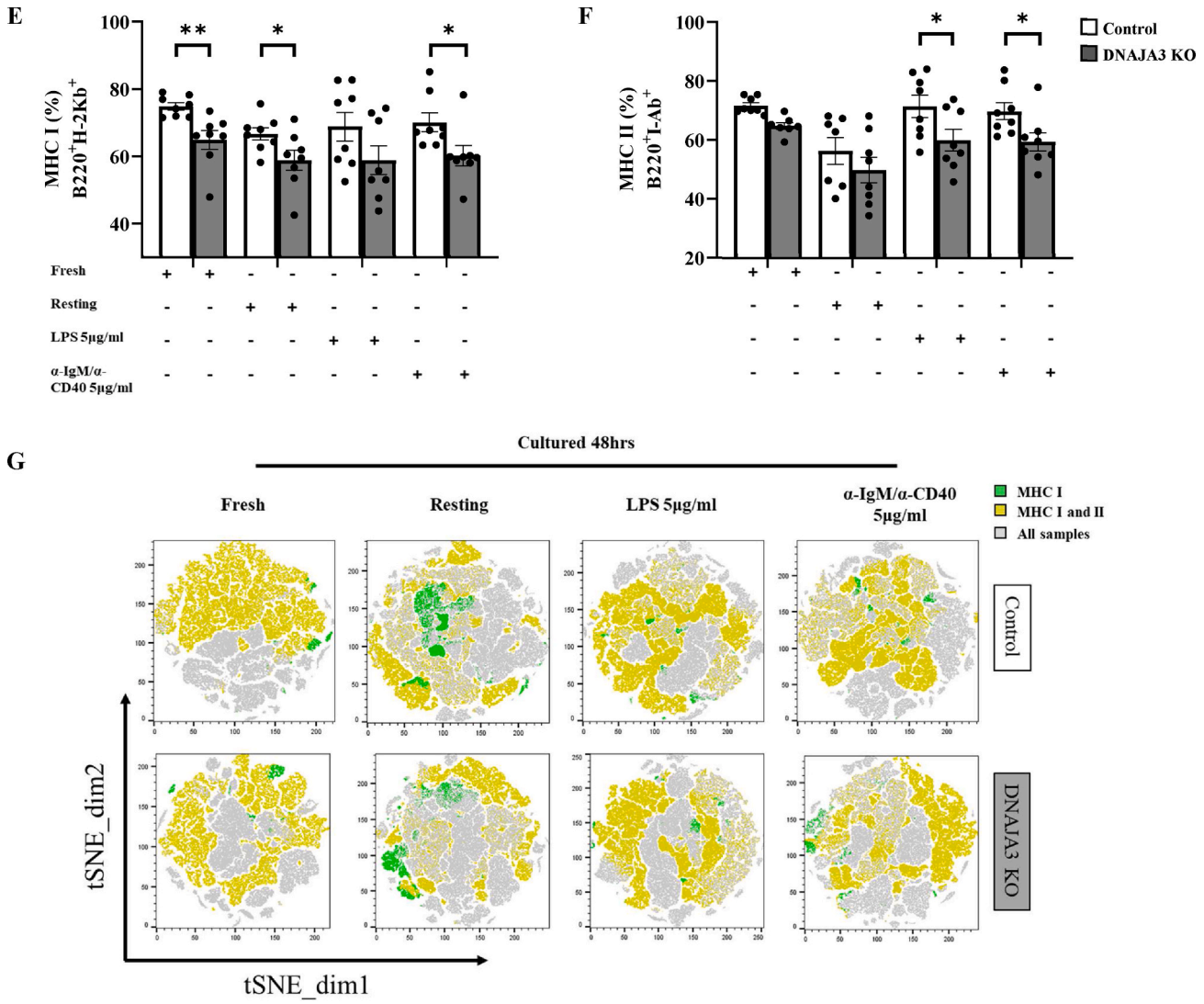


Fig. 6. A–G. Decreased co-stimulatory expressions in activated B cells of DNAJA3 KO mice. The expression profile of co-stimulatory markers on splenic B cells was examined by flow cytometry. $B220^+$ cells positive of $CD40^+$, $CD80^+$ and $CD86^+$ co-stimulatory markers decreased in DNAJA3 KO regardless of TD or T1 antigen stimulation **(A–C)**. DNAJA3 KO mice showed reduced expression of MHC I and MHC II receptors regardless of antigen stimulation condition **(E–F)**. Overlay of $B220^+$ cells of co-stimulatory molecules and MHC I and MHC II tSNE plot between Control and DNAJA3 KO in different stimulation conditions **(D, G)**. The symbol * as $p \leq 0.05$ and ** as $p < 0.01$ in comparison to control mice.



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no antigen and with TD antigen stimulation. In contrast, no difference exists in MZ B cells with or without antigen stimulation (Fig. 5A–B). Results suggest that DNAJA3 KO mice favor an innate humoral response but may lack the ability to have sustained or prolonged immune protection from high-affinity pathogen-specific antibodies produced by FO B cells with the help of T cells. To study B cell activation, we examined the expression of early (CD69) and late (CD25) activation markers in $B220^+$ cells stimulated with TI and TD antigen. Expression of $CD69^+$ or $CD25^+$ did not differ much in LPS stimulation (data not shown), but a visible reduction of $CD69^+$ and a significant decrease of $CD25^+$ cells ($p < 0.005$) in TD antigen stimulation can be seen in DNAJA3 KO mice (Fig. 5C). CD25 is known to be upregulated in activated B cells in secondary lymphoid organs and are suggested to be efficient in antigen presentation [23].

Reduced co-stimulatory molecules in B cell activation of DNAJA3 KO mice

A robust antibody response of activated B cells requires interaction with T cells. The significant reduction of FO B cells in DNAJA3 deficient mice suggests that T-dependent immune response in DNAJA3 KO mice is comprised and that co-stimulatory molecules involved in the process are reduced. To investigate this, we checked for the expression of CD40, CD80, CD86, MHC I, and MHC II in activated B cells. As suspected, the expression of several co-stimulatory molecules in DNAJA3 KO mice is lower in both TI and TD antigen stimulation. The difference is significantly noticeable in $CD40^+$ and $CD86^+$ B cells in TD antigen stimulation (Fig. 6A, 6C-D). In connection, MHC I and II expression are also reduced regardless of the kind of antigen stimulation (Fig. 6E–G). MHC II expression in DNAJA3 KO mice significantly decreased in TI and TD antigen stimulation. MHC II is essential in B cells in forming a complex with the peptide derived from bound antigens. This peptide-MHC II complex is recognized by T helper cells that signal survival and induced proliferation of B cells. Data suggests that co-stimulation function in B cells of DNAJA3 deficient mice during TD antigen stimulation might be sub-optimal based on the reduced expression of co-stimulatory molecules.

DNAJA3 deficiency impaired the functional execution of mature B cells

An immune response consists of the active proliferation of lymphocytes. Activated B cells, such as germinal center B cells, undergo intense proliferative activity that eventually differentiates into antibody-secreting cells and plasma cells. We examined the proliferation of activated B cells by CFSE and MTT proliferation assay. CFSE proliferation assay of LPS-stimulated splenocytes after 48 h showed a significant difference in proliferation index (PI) between control and DNAJA3 KO (Fig. 7A–B, $p = 0.046$). Meanwhile, MTT assay results showed a significant decrease of $OD570_{nm}$ in TI ($p = 0.041$) and TD ($p = 0.034$) antigen

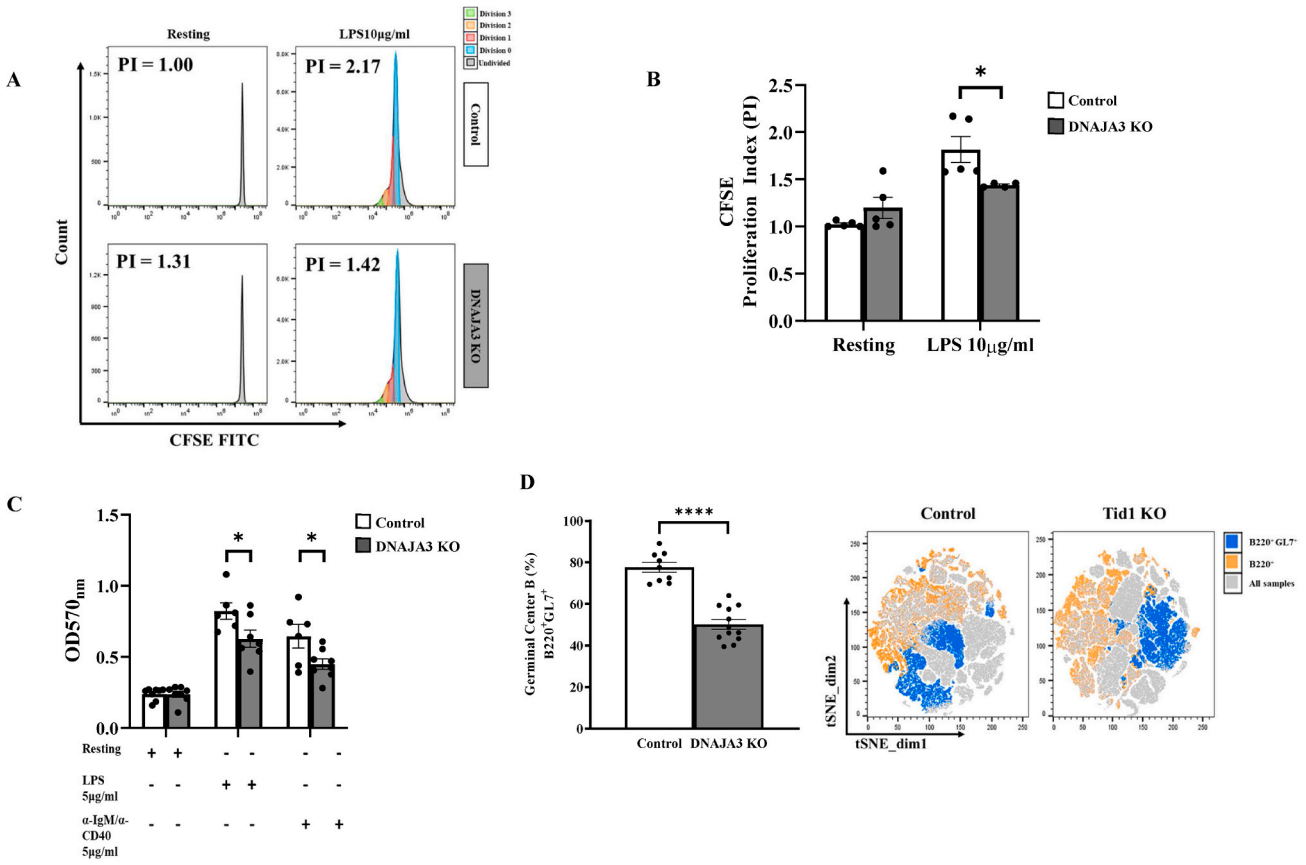


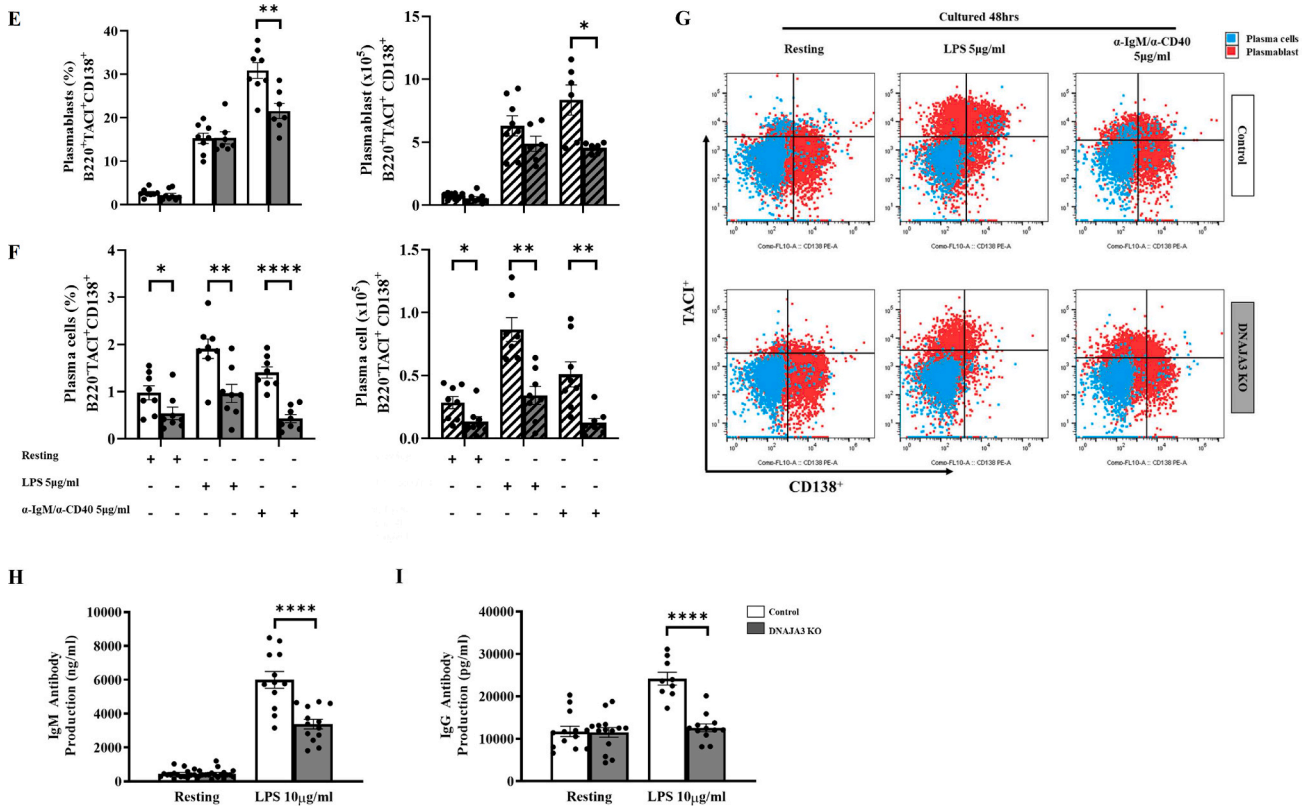
Fig. 7. A–I. Compromised immune response in activated B cells of DNAJA3 KO mice. Splenocytes cultured with LPS 10 μg/ml for 48 h was collected and stained with CFSE for proliferation analysis. Proliferation after 48 h showed mildly significant proliferation index (PI) between Control and DNAJA3 KO mice **(A–B).** MTT cell proliferation assay showed significant reduction of mitotic cells in DNAJA3 KO mice whether stimulated with TI or TD antigen stimulation **(C).** Germinal B cells ($B220^+GL7^+$) present in activated B cells showed significantly decreased in DNAJA3 KO. While population distribution of germinal center as seen in tSNE plot did not show much difference between Control and DNAJA3 KO mice **(D).** Splenocyte cells (1×10^6) were cultured in 96 well plate treated with or without LPS 5 μg/ml (TI-1 antigen) and α-IgM/α-CD40 5 μg/ml (TD antigen) for 48 h. Cells are stained with $B220^+$, $TACI^+$, $CD138^+$ antibody to distinguish plasmablast ($B220^+TACI^+CD138^+$) and plasma cells ($B220\text{-}TACI^+CD138^+$) in flow cytometry analysis. Cell percentage and cell number of plasmablast showed a significant reduction in TD antigen stimulation of DNAJA3 KO mice versus Control. Subsequently, plasma cell percentage and cell number clearly showed a significant reduction in DNAJA3 KO mice in both TI and TD antigen stimulation **(E–F).** Overlay of plasmablast and plasma cells of control and DNAJA3 KO mice in different antigen stimulation **(G).** Supernatant of activated B cells (LPS 10 μg/ml; 48 h) was collected and analyzed by Ig-ELISA. In DNAJA3 KO mice, the production of IgM and IgG antibodies from ex-vivo culture are significantly reduced compared to control mice **(H–I).** The symbol * as $p \leq 0.05$, ** as $p \leq 0.01$ and **** as $p \leq 0.0001$ in comparison to control mice.

stimulation treatment in DNAJA3 deficient mice (Fig. 7C). Similarly, germinal center B cells ($B220^+GL7^+$) percentage showed a significant reduction in DNAJA3 KO (Fig. 7D). Germinal B cells subsequently differentiate into plasmablast ($B220^+TACI^+CD138^+$) and plasma cells ($B220^-TACI^+CD138^+$). The significant reduction of germinal B cells in DNAJA3 KO mice subsequently reduced germinal B cells' differentiation into plasmablast (cell number and percentage) in DNAJA3 KO mice activated with TD antigen (Fig. 7E and G). Plasma cell populations in activated B cells, whether by TI or TD antigen stimulation, showed a significant reduction in DNAJA3 KO mice (Fig. 7F–G) which resulted in less production of IgM and IgG antibody (Fig. 7H–I, $p \leq 0.0001$). Results suggest that the proliferative activity of DNAJA3 KO mice is not only hampered by DNAJA3 deficiency but also differentiated B cells involved in antibody production, such as plasmablast and plasma cells, are affected in DNAJA3 deficient mice and that immune response in DNAJA3 KO mice is compromised.

DNAJA3 deficiency in B cells results in mitochondria content and functional abnormalities

B cells undergo metabolic shifts necessary for cell proliferation and differentiation throughout their development. Maintaining their mitochondria activity and function is essential to their eventual success in producing antibody-secreting B cells during an immune response. Since DNAJA3 can regulate mitochondria function thus, the mitochondrial content and function of activated B cells in control versus DNAJA3 KO mice were investigated in order to clarify whether the state of mitochondrial activity is associated with reduced B cell development and function in DNAJA3 deficiency mice. We utilized a series of fluorescent-based mitochondrial function assays using flow cytometry techniques described by Monteiro et al. [24]. The result in mitochondria mass analysis showed a significant decrease in TD stimulation ($p = 0.035$, Fig. 8A–B), while mitochondrial membrane potential showed an overall reduction in DNAJA3 KO mice, especially in TD antigen stimulation ($p = 0.014$, Fig. 8C–D). Functional mitochondria in $B220^+$ B cells (indicated by Mitotracker $Green^{+/high}$ Mitotracker $Red^{+/high}$ in quadrant of panel 8 E) showed overall low in DNAJA3 KO mice without or with both TI or TD antigen stimulation (Fig. 8E–F). Conversely, dysfunctional mitochondria in $B220^+$ B cells (indicated by Mitotracker $Green^{+/high}$ Mitotracker $Red^{-/low}$ in quadrant of panel 8 E) consistently increased in resting and two types of stimulation in DNAJA3 KO mice, noticeably in LPS stimulation ($p < 0.05$, Fig. 8E, G).

Metabolic demands of B cells change significantly during development and antigen stimulation, which require sufficient ATPs.



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Mitochondria are known to generate ATP via oxidative phosphorylation (OXPHOS) complexes. Therefore, we further investigated the OXPHOS complex protein expression between control and DNAJA3 KO mice from isolated B cells. OXPHOS complex protein expression in DNAJA3 KO mice significantly decreased compared to the control (Fig. 8H–I). Complex I (NDUFBS) protein expression in DNAJA3 KO mice showed drastic reduction together with complex II (SDHB) and complex IV (MTCO1) but not complex V (ATP5A) (Fig. 8H–I). Results show that DNAJA3 deficiency attenuates the function of mitochondria by loss of mitochondria complex proteins involved in ATP synthesis, decreased mitochondria mass, and reduced membrane potential.

Discussion

DNAJA3 is a heat shock protein 40 under the DNAJ protein family (HSP40/DNAJA3) that can function independently from HSP70 [25]. Our study indicates that DNAJA3 can influence B lymphocyte development and function based on our DNAJA3-deficient B cell mice model. There is an overall attenuation of the $B220^{+}$ B cell population in the bone marrow, spleen, blood, MLN, and PP and early B cell developmental stages from Pre-B to Immature B cells in the bone marrow of DNAJA3 KO mice. Consequently, Immature and Mature B cells are reduced, notably in the spleen, blood, and lymph node. DNAJA3-deficiency also affects the diverged outcome of B1 and B2 progenitor development. DNAJA3 KO mice in the adult stage showed a dramatic decrease in peritoneal B1 cells, particularly B1b subsets. The B1 cells initiate a rapid innate immune response similar to marginal (MZ) B cells in T cell-independent manner [16]. B1 cells are known to secrete natural antibodies after antigen exposure but without differentiation into plasma cells [26]. Also, they secrete antibodies naturally (B1a) or from exposure to antigens (B1b) [17,27]. B1b cells differ from B1a due to their ability to form memory B cells [28] and recognize protective antigens in bacteria [26]. B1a cells are produced during early fetal development and maintain self-renewal in the peritoneal cavity. Meanwhile, B1b is maintained by progenitors in the bone marrow and repopulates in adult bone marrow [29]. Despite the B1 cell's ability to self-renew, peritoneal B1b cells in DNAJA3 deficient mice are significantly reduced along with reduced total B cells in the bone marrow. It has been reported that galectin-3 plays a role in regulating peritoneal B1-cell differentiation into plasma cells, confirmed by galectin-3 KO mice [30]. Later, Chen et al. [13] identified galectin-7 as one DNAJA3/Tid1-interacting client protein in human head and neck squamous cell carcinoma. In the present study, we did not investigate the action mechanism of how DNAJA3 proteins cause the reduction of B1b lymphocytes. Therefore, how DNAJA3 regulated B1-cell development requires further study.

Conventional B (B2) cells are crucial in launching antibody response during adaptive immunity by producing multitudes of antibodies capable of eliminating pathogens and toxins [31]. Hence, B2 cell development and function are crucial in mammalian adaptive immunity. We showed in our results that disruption of early B-cell development stages could already be detected during Pro B cell stages that significantly affected the succeeding later development stages in DNAJA3 deficient mice compared to control mice. It has been demonstrated that DNAJA3/Tid1 can regulate DN3 thymocytes towards the double-positive stage via increasing IL-7-related signaling pathways such as IL7R, Jak3, and bcl-2 [12]. The same signaling mechanism is also crucial in early B2 lymphocyte development. Furthermore, it has been reported that DNAJA3 has a regulatory function in the Jak/STAT signaling pathway, which is vital for the growth and development of mammalian tissues, especially the hematopoietic lineages [6]. Peripheral B2 B cell populations are subdivided into follicular (FO) B cells in the major and marginal (MZ) B cells in the minor. They are known to respond rapidly to blood-borne pathogens [32]. FO B cells in follicles can recirculate throughout the secondary lymphoid tissues (e.g., lymph nodes, spleen) close to the T cell zones, allowing them to present T-dependent antigens to activated T cells. MZ and B1a B cells are considered to have similar functions as the innate-like immune response

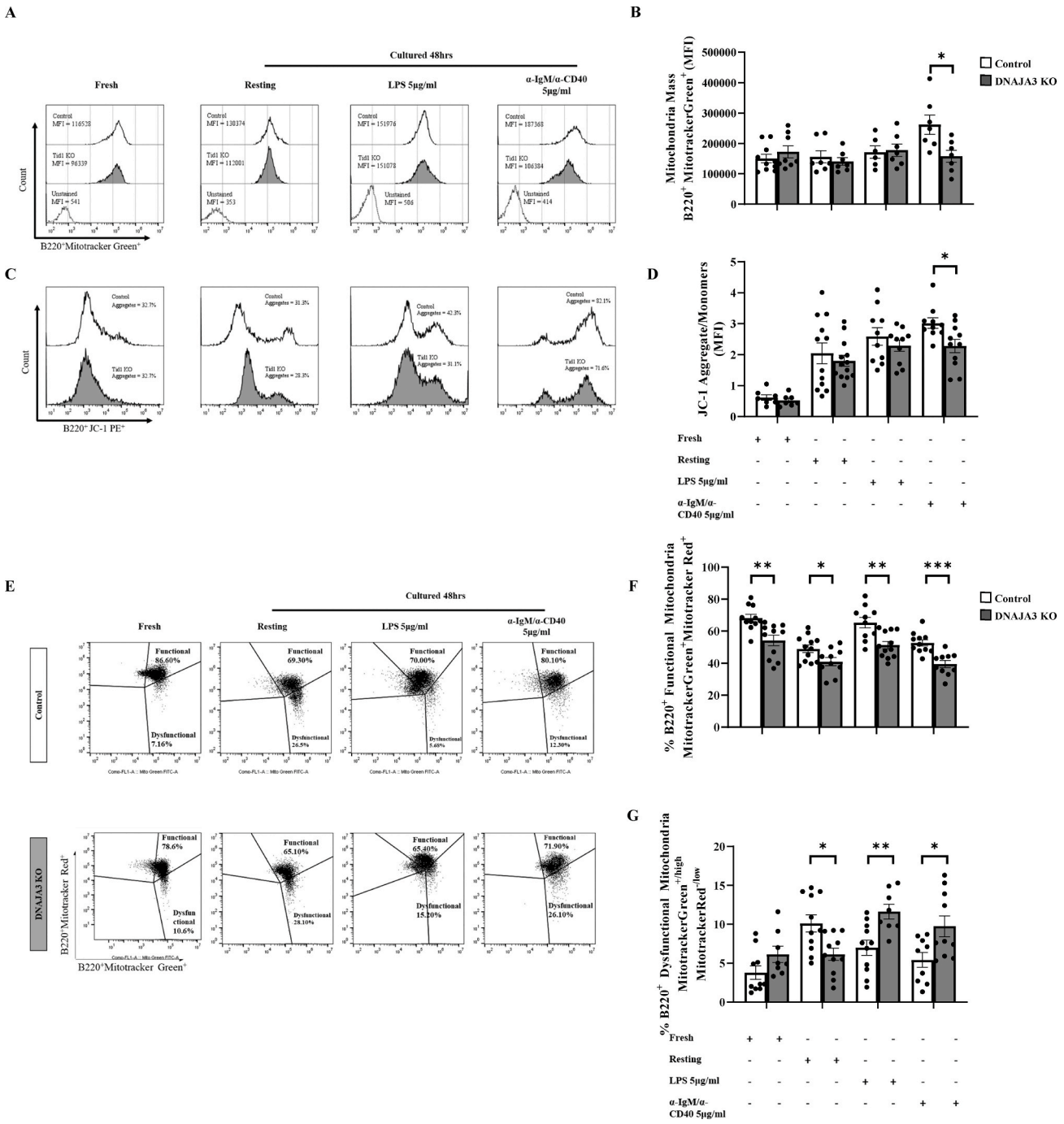

Fig. 8. A–I. Dysfunctional mitochondria and low OXPHOS protein complex expression in B cells of DNAJA3 KO mice. Splenocyte cells were collected from control and DNAJA3 KO mice. RBC lysed splenocytes were cultured in 96 well plate with or without LPS 5 μg/ml (TI-1 antigen) or a combination of α-IgM and α-CD40 5 μg/ml (TD antigen) for 48 h. Cells were then stained with $B220^+$ antibody, then cells stained with Mitotracker Red and Green following manufacturing protocol. Mitochondria mass (A–B) and membrane potential (C–D) significantly decreased in TD antigen stimulation in DNAJA3 KO mice. Functional mitochondria of $B220^+$ cells (Mitotracker Red^+ Mitotracker $Green^+$) is significantly impaired in DNAJA3 KO mice for both antigen stimulation (E–F), in relation, dysfunctional mitochondria of $B220^+$ cells (Mitotracker $Red^{-/low}$ Mitotracker $Green^+$) increased in TD or TI antigen stimulation in DNAJA3 KO mice compared to Control (E, G). To further examine the OXPHOS respiratory complex, $B220^+$ B cells sorted from RBC lysed splenocytes. The purity of B cells after sorted was verified by flow cytometry and showed 95–98% purity. The protein extracts were prepared and the expression level of OXPHOS respiratory complex proteins was evaluated by immunoblot. In addition, β-actin was used as loading control in immunoblot assay. OXPHOS protein complex expression in B cells clearly show a reduced expression of mitochondria complex proteins in DNAJA3 KO mice compared to control. Complex I drastically reduced in DNAJA3 KO mice similarly in complex II and complex IV (H–I). As

of B lymphocytes, while B1b cells contribute to adaptive immunity [17, 27]. MZ B and B1 B cell activation depend on co-stimulation, in contrast to FO B cells' dependency on T cells to produce antibodies [16]. Like B1, MZ B cells can limitlessly self-renew in contrast to FO B cells [33]. DNAJA3 KO mice significantly showed low FO B cell distribution during TD stimulation but a high MZ B cell population during TI stimulation

shown in overlay histogram plot and mean fluorescence intensity (MFI) bar graphs, the symbol * as $p \leq 0.05$, ** as $p \leq 0.01$ and *** as $p \leq 0.001$ in comparison to control mice.

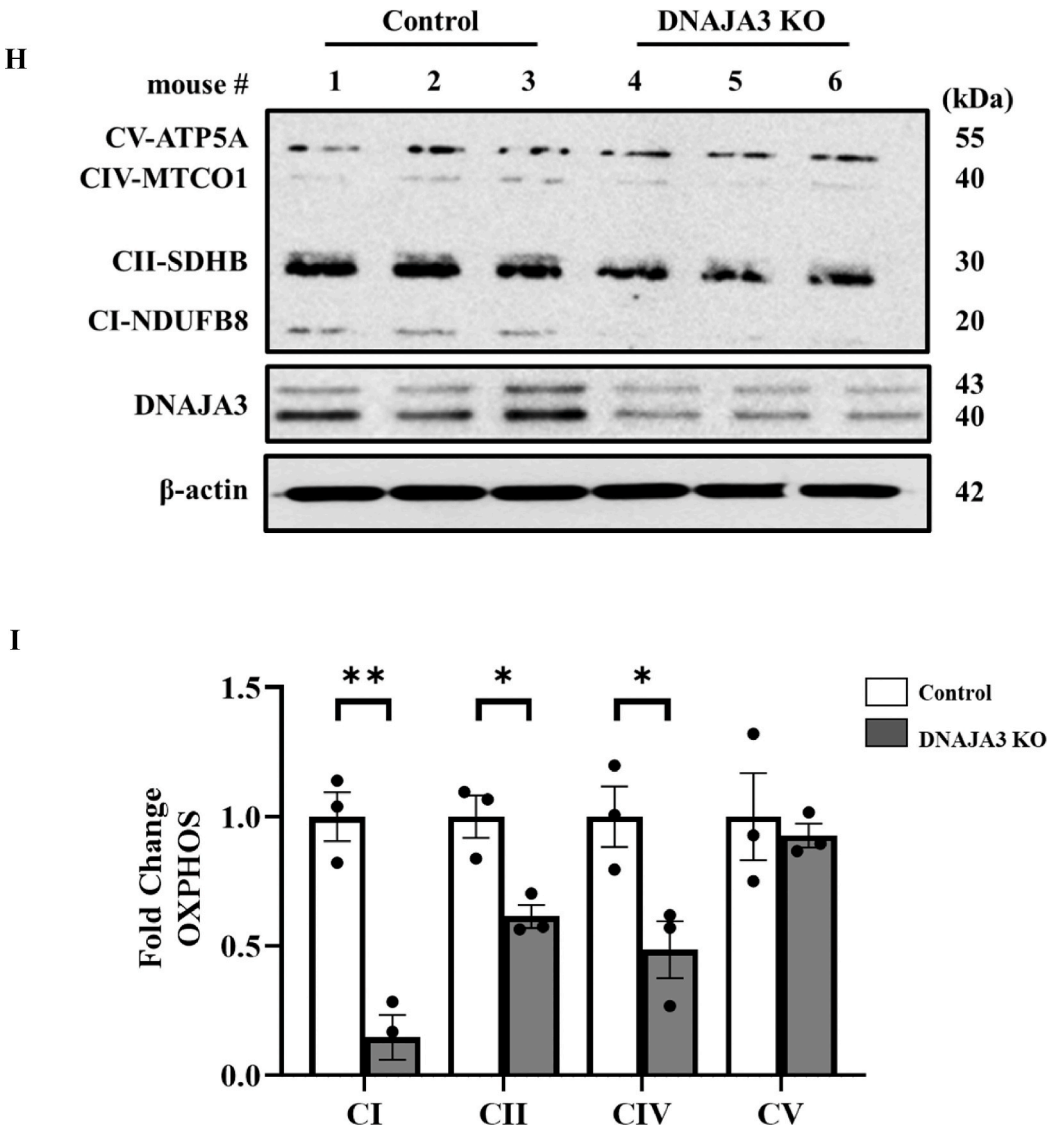

Fig. 8. (*continued*).

compared to control mice. Concomitantly, B1 cell subpopulations showed a significant decrease of B1b cells compared to the B1a cell population in DNAJA3 KO mice. It does imply that DNAJA3 KO mice favor an innate fast response as it favors MZ and B1a cell populations and that long-term adaptive immunity in DNAJA3 KO mice might be compromised.

Co-stimulatory signals are essential for activating naïve lymphocytes effectively. The maturation of B cells through cognate T cell interaction requires the expression of co-stimulatory molecules, including CD40, CD80, CD86, and MHC II. CD40 in B cells is analogous to CD28 in T cells as both receptors augment Akt activation, which is crucial for cell survival, cell cycle progression, glucose uptake, and metabolism in activated B cells. Additionally, the B7 family of proteins, CD80 (B7.1) and CD86 (B7.2), and MHC II, are significant co-stimulatory molecules in B cells, involved in their antigen presentation cell function. CD80 and CD86 can activate T cells through engagement between CD28/CD80 or CD28/CD86. In our study, DNAJA3 KO mice exhibit decreased FO B cell population, suggesting an attenuated T-dependent immune response. Furthermore, the significant decrease in co-stimulatory molecule expression during TD antigen stimulation of $CD40^{+}$, $CD86^{+}$, and $MHC\ II^{+}$ B cells supports the attenuated T-dependent immune response hypothesis in DNAJA3 KO mice. Activated FO B cells can further differentiate into GC, which can differentiate into short- or long-lived plasma cells. Our results demonstrated that the percentages and cell numbers of plasmablasts and plasma cells significantly decreased in DNAJA3 KO mice and decreased FO B cells in GCs. The proliferative ability of DNAJA3 KO mice, when activated, is affected, as shown in CFSE and MTT assay, which in turn resulted in a significant reduction of antibody-producing cells, such as plasma cells, that contributed to a decrease in IgG and IgM antibody production.

DNAJA3 can regulate mitochondria function. Thus, we investigated the mitochondrial mass, membrane potential, and function of activated B cells in DNAJA3 KO mice. Our results strongly supported that DNAJA3 is essential in functional mitochondria maintenance. B cells undergo metabolic shifts that are necessary for antigen challenge. B cell metabolic processes undergo significant modifications, leading to activation and subsequent initiation of an immune response. Oxidative phosphorylation (OXPHOS) and glycolysis increase rapidly in B cells upon antigen stimulation. However, during the initial few hours of antigen stimulation, the processing of the antigen for presentation relies exclusively on

OXPHOS, as there is no increase in glucose uptake. This initial bias towards OXPHOS during B cell activation results in a higher demand for mitochondrial mass in activated B cells [19,34,35]. According to Lu et al. [36], the DNAJA3 protein in mitochondria utilizes its DnaJ domain to stimulate the ATPase activity of mitochondria HSP70 (mtHSP70). Its localization in the matrix is crucial for the folding and degradation of mitochondrial proteins and the maintenance of mitochondrial DNA. Surprisingly, mitochondria respiratory complex I, II, and IV protein expression was significantly decreased in DNAJA3 KO mice. The loss of mitochondria complex protein might be associated with the loss of DNAJA3's essential role in mitochondrial membrane potential maintenance and mitochondria DNA integrity. It has been shown that DNAJA3/Tid1 associates with mtDNA for its maintenance, but when ATP synthesis is inhibited, DNAJA3/Tid1 is recruited by complex I, protein aggregation occurs, and mtDNA is lost, which in turn results in loss of mitochondria function [37].

Our findings suggest that the observable attenuation of various B cell populations in an early and activated state may be due to a dysfunctional mitochondrial profile in DNAJA3 KO mice. Specifically, our results indicate that B cells in DNAJA3 KO mice exhibit increased dysfunctional mitochondria activity while a significant decrease in mitochondrial protein complexes, mitochondria mass, and membrane potential, particularly during TI or TD antigen stimulation.

Conclusions

Our study has provided a broader perspective on the role of DNAJA3 in B cell development and their function execution. Through investigating different B cell populations and subsets, we have demonstrated that DNAJA3 deficiency can weaken the development and function of B cells in primary and secondary immune organs. DNAJA3 might be an immune regulator in B lymphocyte development. The intricate process of B cell proliferation and differentiation involves various factors such as cell surface receptors, intracellular signaling molecules, and transcription factors [38]. DNAJA3 has been previously reported to affect the signaling pathway of kinases involved in tumorigenesis as a co-chaperone and downregulate kinase activities associated with tumor progression [39]. In the present study, DNAJA3 is essential in maintaining normal mitochondria function in B lymphocytes. However, its role as an immune regulator in lymphocyte development and activation remains largely unexplored.

Ethics approval and consent to participate

All animal operations follow standard laboratory animal procedures following the animal management and rearing rules in "Guide for Care and Use of Laboratory Animals" (NRC1996-2011). Animal use is approved by the Institutional Animal Care and Use Committee (IACUC) of the Fu-Jen Catholic University (IACUC No. A10713).

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. Further inquiries should be directed to the corresponding authors.

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Declaration of competing interest

All authors have no conflicts of interest to declare that pertains to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bj.2023.100628.

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