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Lupus exacerbation in ovalbumin-induced asthma in Fc gamma receptor IIb deficient mice, partly due to hyperfunction of dendritic cells

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Abstract

Background: Although allergy might be another factor that exacerbates lupus as demonstrated by several epidemiologic studies, the direct correlation between lupus activities and allergy is still in question.

Objective: To explore the correlation between allergic reaction and lupus activities.

Methods: The allergic asthma model using ovalbumin (OVA) administration in wildtype (WT) and Fc gamma receptor IIb deficient (FcgRIIb-/-) mice (a lupus-prone model) together with *in vitro* experiments on bone marrow-derived dendritic cells (DCs) were performed.

Results: At 2-weeks-post OVA, both WT and FcgRIIb-/- mice demonstrated similar allergic reaction as indicated by an elevation of IgE and IL-4 in serum with asthma-liked lung histology (lung weight, inflammatory score, and bronchial thickness) with increased spleen weight. Apoptosis in the lungs and spleens (activated caspase 3 immunohistochemistry) was detected only in OVA-administered FcgRIIb-/- mice. Surprisingly, OVA-administered FcgRIIb-/- mice, demonstrated active lupus nephritis, as indicated by anti-dsDNA, proteinuria, and renal immune complex deposition (immunohistochemistry analysis) implying an impact of allergy on lupus activities. Meanwhile, serum creatinine and gut permeability defect (FitC-dextran assay and endotoxemia) were not different between the FcgRIIb-/- mice with OVA versus with control. In parallel, FcgRIIb-/- DCs were more susceptible to activations by OVA and lipopolysaccharide (LPS) than WT DCs as demonstrated by CD80 with major histocompatibility complex II (MHC II) using flow cytometry analysis.

Conclusions: OVA-induced allergy in FcgRIIb-/- mice exacerbated lupus activity, possibly due to hyper-responsiveness of FcgRIIb-/- DCs over WT from the loss of inhibitory FcgRIIb. The proper control of allergy might be beneficial for lupus.

Key words: Ovalbumin, lupus, Asthma, FcgRIIb, Dendritic cells, T cells

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Introduction

Systemic lupus erythematosus (SLE) is a common autoimmune disease with multi-organ dysfunctions due to the deposition of circulating immune complexes in several organs.[1] The pathogenesis of SLE consists of environmental factors and genetic defects. Despite several reports on genetic causes, the dysfunction polymorphism of the Fc gamma receptor IIb (FcgRIIb) is quite common in Asian populations.[2] Interestingly, FcgRIIb is the only inhibitory receptor in the FcgR family. As such, mice with FcgRIIb deficiency (FcgRIIb-/-) demonstrate lupus characteristics, and have been used as a representative mouse model of SLE.[3-5] The loss of inhibitory FcgRIIb enhances the reaction against molecules either from pathogens or host cells, partly due to the crosstalk between FcgRs and several receptors.[6-8] Indeed, several activators induce hyper-inflammatory responses in FcgRIIb-/- mice, including drugs, particulate matter 2.5, endotoxin, lipids, and fungal molecules.[9-14] The hyper-inflammatory responses can exacerbate lupus activity as demonstrated in acute viral infection and other conditions with inflammation-induced apoptosis, including silica dust, ultraviolet light, and other strong immune activators.[15-17] Notably, apoptosis might be a source of autoantigens recognized by anti-dsDNA, a specific autoantibody in lupus forming the immune complexes between anti-dsDNA and nucleic acid molecules, which is a common component of the lupus pathogenesis.[18]

Among several lupus activating factors, allergy might be another factor that exacerbates lupus. Indeed, there are i) nearly 60% of the patients with active lupus demonstrate concurrent allergic symptoms,[19] ii) a higher rate of atopic dermatitis in patients with lupus,[20] and iii) the 1.4-fold increased risk of lupus among patients with allergic rhinitis from a meta-analysis report.[21] However, a direct study on the correlation between lupus activity and allergy is still lacking. Because of the age-dependent lupus characteristic in FcgRIIb-/- mice as indicated by the spontaneous elevation of serum anti-dsDNA as early as 16–24 wks old, FcgRIIb-/- mice younger than 16 wks old are used as asymptomatic lupus mice.[22,23] In parallel, ovalbumin (OVA)-induced allergic asthma is a well-known IgE-mediated hypersensitivity mouse model in either BALB/c or C57BL/6 mice.[24,25] Hence, we compared OVA-induced asthma in young asymptomatic lupus-prone mice from wildtype (FcgRIIb+/+) and FcgRIIb-/- groups together with the *in vitro* experiments on dendritic cells and T cells.

Materials and Methods

Animals and animal model

C57BL/6 mice (WT) mice (8 weeks old) were purchased from Nomura Siam International (Pathumwan, Bangkok, Thailand), and FcgRIIb deficient mice with a C57BL/6 background (FcgRIIb-/-) were provided by Dr. Silvia Bolland (NIAID, NIH, Maryland, USA). The study protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, following the National Institutes of Health (NIH), USA. For ovalbumin-induced allergic model, 8-week-old mice were sensitized by intraperitoneal injection of ovalbumin (OVA) 50 µg/dose (Sigma-Aldrich, St.Louis, MO) with complete Freund's adjuvant (CFA, 1 mg; Chondrex, Woodinville, WA, USA) emulsified in sterilized phosphate-buffered saline (PBS) on day 0 and again on day 7 (**Figure 1A**). All procedures were performed in a sterile environment. On day 14, mice were sacrificed with cardiac puncture under isoflurane anesthesia.

Mouse sample analysis and gut permeability determination

Spot urine collection was performed by placing mice in the metabolic cage (Hatteras Instruments, NC, USA) for a few hours in each time point and before sacrifice. For quantitative determination of creatinine and urine protein, QuantiChromTM Creatinine-Assay (DICT-500) (BioAssay, Hayward, CA, USA) and Pierce™ BCA Protein Assay Kit (Thermo Scientific, Wilmington, DE, USA), respectively, were used. Serum cytokines and IgE were measured by ELISA (Invitrogen, Carlsbad, CA, USA), while serum anti-dsDNA was analyzed following a previously published protocol using coated Calf-DNA (Invitrogen, Carlsbad, CA, USA).[26] Gut permeability was determined by fluorescein isothiocyanate-dextran dextran (FITC-dextran) assay and together with the spontaneous detection of lipopolysaccharide (LPS or endotoxin) in blood.[27-29] As such, the detectable FITC-dextran (a non-intestinally absorbable carbohydrate) in serum after an oral gavage or the elevated LPS in blood without active infection indicate a gut permeability defect. Accordingly, FITC-dextran (FITC-dextran; molecular weight 4.4 kDa) (Sigma-Aldrich, St. Louis, MO, USA) at 25 mg/mL (0.5 mL) was orally administered for 3 h before sacrifice and serum FITC-dextran was measured with fluorospectrometry (Thermo Scientific, Wilmington, DE, USA) with the excitation and emission wavelength at 485 and 523 nm, respectively, using a standard curve from serially diluted FITC-dextran. Meanwhile, LPS was measured by HEK-Blue LPS detection (InvivoGen, San Diego, CA, USA).

Histology and immunofluorescence

The organs were fixed in 10% formalin, embedded in paraffin, cut to a 4 µm thickness, and stained with Hematoxylin-Eosin (H&E) color. The evaluation of lung injury was performed following previous publications.[24,25] Briefly, a subjective scale of 0 to 4 based on the inflammatory cell infiltration per high power field of the lung histopathology (HPF; 400× magnification) using 10 HPFs per mouse, including 0: no inflammatory cells, 1: few cells, 2: the inflammatory cells in 1 cell layer deep; 3: the inflammatory cells in 2-4 cells deep, 4: the inflammatory cells of > 4 cells deep. For liver injury evaluation, the score based on cell congestion, cellular degeneration, cytoplasmic vacuolization, leukocyte infiltration, and cellular necrosis, in 10 randomly selected fields at 200× magnification for each animal with the following score of the damaged area per the examined field: 0 areas less than 10%; 1 damage 10-25%; 2 damage involving 25-50%; 3 damage 50-75%, and 4 indicates 75-100% of the area being affected,

following previous publications[30] was used. In parallel, the kidney injury score based on tubular epithelial swelling, loss of brush border, vacuolar degeneration, necrotic tubules, cast formation, and desquamation using the following scoring method: 0, area of damage < 5%; 1, area of damage 5–10%; 2, area of damage 10–25%; 3, area of damage 25–50%; and 4, area of damage > 50%, as previously published.[31] For apoptosis detection in several organs, immunohistochemistry with an anti-active caspase-3 antibody (Cell Signaling Technology, Beverly, MA, USA) was used. The apoptotic cells per slide were counted at 200× magnification and expressed as positive cells per high-power field as previously published.[32] Additionally, mouse organs from both samples were put in Cryogel (Leica Biosystems, Richmond, IL, USA) (stored at -80°C) and 10% formalin for fluorescent imaging and histology analysis (Hematoxylin and Eosin staining), respectively. The immunoglobulin deposition in kidneys was visualized by immunofluorescence prepared in Cryogel, stained with goat anti-mouse IgG and DAPI (4′,6-diamidino-2-phenylindole), a blue-fluorescent DNA stain (Alexa Fluor 488, Abcam, Cambridge, MA, USA), then detected and analyzed for fluorescent intensity by ZEISS LSM 800 (Carl Zeiss, Germany).

Bone marrow-derived dendritic cells and the in vitro experiment

Bone marrow-derived dendritic cells (DCs) from mouse long bones (tibia and femur) of 8-week-old wildtype (WT) and FcgRIIb-/- mice following a previous publication were used.[33] Briefly, following red cell lysis, cells were grown in RPMI 1640 medium supplemented with 10% FBS, Sodium Pyruvate (1 mM), HEPES (10 mM), L-Glutamine (1×), MEM NEAA (1×), Pen-Strep (100 Units/ml), GM-CSF (20 ng/ml) and IL-4 (20 ng/ml) at a density of 1×10^6 cell/ml. Then, the cells were incubated at 37°C in 5% CO_2 with fresh media changing every two days. After cultures for 6 days, the immature DCs were stimulated with OVA (100 μg/ml) and LPS (1 μg/ml) for 24 h before supernatant and cell collection for additional analysis. An optical inverted-phase contrast microscope was used to dynamically observe the morphological changes occurring in the culture system's cells on a daily basis. The purity of DCs was flow cytometrically detected. On day 7 after the suspending cells were harvested, the flow antibody Alexa Fluor® 647 anti-mouse CD11c Antibody, PE/Cyanine7 anti-mouse CD80 antibody, and FitC anti-mouse I-A^b Antibody (Biolegend, San Diego, CA, USA) were added according to the instructions and detected on flow cytometry. The naïve CD4+ T cells were isolated from the spleen using Mouse Naïve CD4 positive (CD4+) T cell isolated kit (Stemcell, Carlsbad, USA), and flow cytometric antibodies APC anti-mouse CD3ε Antibody and Alexa Fluor® 488 anti-mouse CD4 Antibody (Biolegend, San Diego, CA, USA) were added to detect the initial T cell purity. After DC stimulation for 24 h, the mature DCs (4×10^4 cells/well) were seeded into a complete medium with naïve T cells (2×10^5 cells/well) for 3 days in 96-well plates coated with Ultra-LEAF™ Purified anti-mouse CD3 Antibody (Biolegend, San Diego, CA, USA). After the co-culture, CD4+ T cells were stained for expression of APC anti-mouse CD3ε Antibody, Alexa Fluor® 488 anti-mouse CD4 Antibody, and PerCP/Cyanine5.5 anti-mouse IFN-γ Antibody (Biolegend, San Diego, CA, USA). All these cell populations were analyzed by flow cytometry using BD LSR-II (BD Biosciences) and the data were analyzed by FlowJo software (Tree Star Inc., Ashland, OR, USA).

Statistical analysis

Mean ± standard error of the mean (SEM) was presented using the Kruskal-Wallis one-way analysis of variance (ANOVA) followed by Siegel-Tukey's analysis for multiple group comparison. Analysis of the time-point data was determined by the repeated measures ANOVA. All statistical analyses were performed with Graph Pad Prism version 10.0 software (La Jolla, CA, USA) and a p-value of < 0.05 was considered statistically significant.

Results

Lupus characteristics in FcgRIIb-/- mice after ovalbumin (OVA)-induced allergic asthma

Asthma was induced by OVA, according to a standard protocol,[34,35] in 8-week-old mice from both wildtype (FcgRIIb+/+) and FcgRIIb-/- groups before the determination of several parameters from blood and internal organs (lungs, livers, kidneys, and spleens) (**Figure 1B-O** and **Figure 2A, B**). As such, after 2 weeks of OVA stimulation, both FcgRIIb+/+ and FcgRIIb-/- mice demonstrated a similar severity of allergic asthma as indicated by retardation of weight gain, elevated serum immunoglobulin E (IgE), increased interleukin-4 (IL-4), and lung parameters (lung weight, lung inflammatory score, lung eosinophils, and bronchial thickness) (**Figure 1B-F, and N-O**). However, apoptosis in the lung was most prominent in FcgRIIb-/- with OVA (**Figure 1G, and O**). For the systemic impact of OVA stimulation in wildtype mice, there was no change in apoptosis in the internal organs (livers, kidneys, and spleens) with only increased spleen weight (**Figure 1H-M**). From the Hematoxylin and Eosin stained slides (**Figure 2A**), histological scores of liver and kidney together with spleen pathological features were not different among groups (data not shown). There was a similar weight of liver and kidney among all experimental groups and spleen weight in control groups of FcgRIIb-/- and FcgRIIb+/+ mice was not different (**Figure 1H, J, and L**). The increased spleen weight after 2 weeks of OVA administration in FcgRIIb+/+ and FcgRIIb-/- mice was also similar (**Figure 1J**). On the other hand, there was apoptosis in livers, spleens, and kidneys of FcgRIIb-/- mice regardless of OVA administration (**Figure 1I, K, M**, and **Figure 2B**) implying impacts of the loss of inhibitory FcgRIIb on apoptosis susceptibility. The abundance of apoptotic cells in livers and kidneys was similar between FcgRIIb-/- with vehicle versus OVA, while OVA more prominently induced spleen apoptosis in FcgRIIb-/- than the control mice (**Figure 1I, K, M**, and **Figure 2B**).

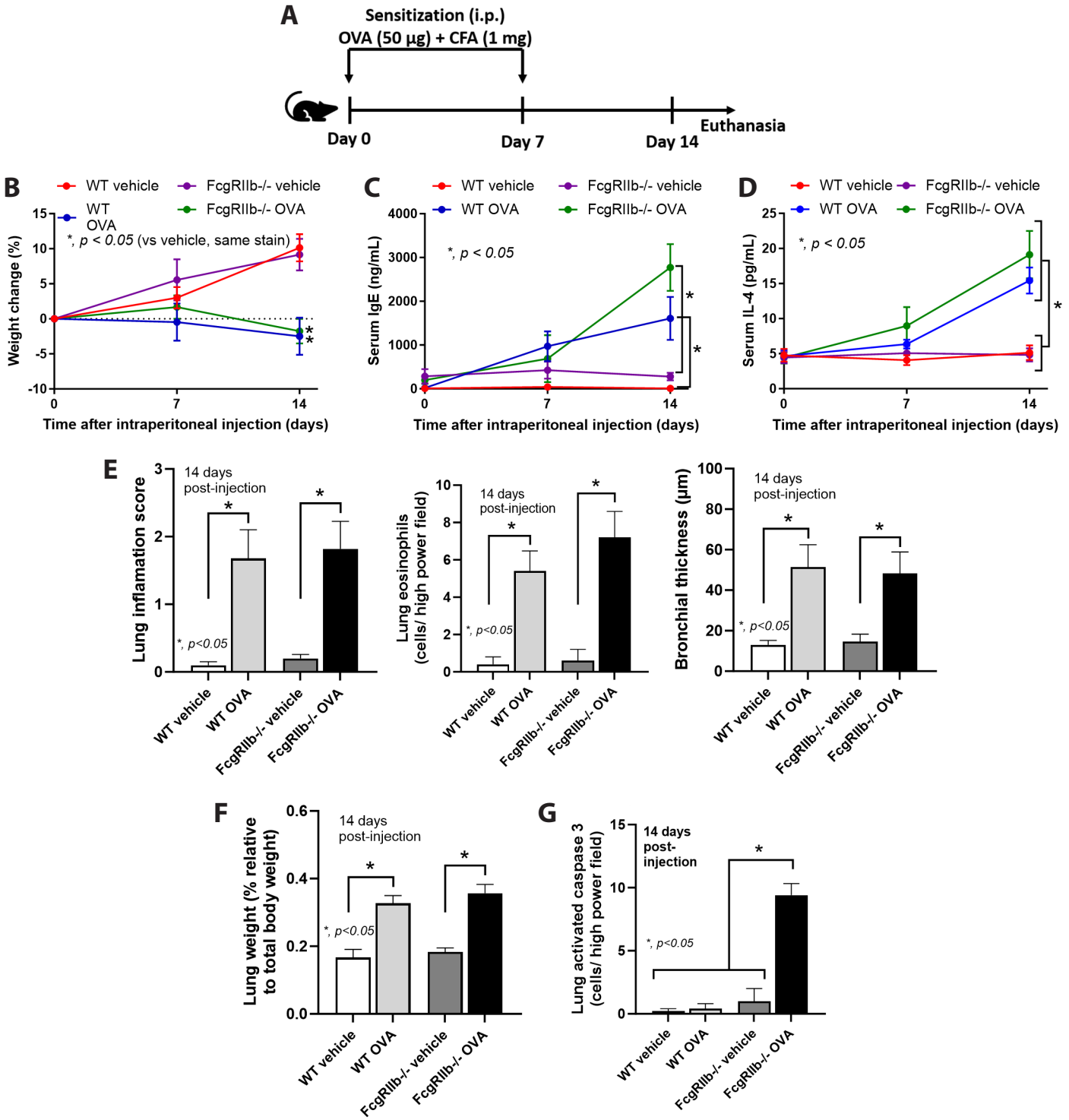

Figure 1. Schema of the establishment of allergic asthma model (see method) is demonstrated (A). Characteristics of wildtype (WT) and FcgRIIb-/- mice with ovalbumin (OVA) or vehicle administration as indicated by the time-point of bodyweight change, serum IgE, and serum IL-4 (B-D) and the parameters at 14-day post-injection as determined by lung injury (lung weight, lung eosinophils, lung inflammation score, bronchial thickness, and activated caspase 3) (E-G), and organ weight with activated caspase 3 of liver (H, I), spleen (J, K), right kidney (L, M) are demonstrated. The representative figures of lung histology of mice with ovalbumin (OVA) or vehicle administration as stained by Hematoxylin & Eosin (H&E) staining (N) and activated caspase 3 immunohistochemistry (O) (original magnification 200×) are demonstrated. Arrow, raw surface of the bronchial thickness (N) and apoptosis (O) are indicated. (n = 5 - 7/group). *, $p < 0.05$ between the indicated groups

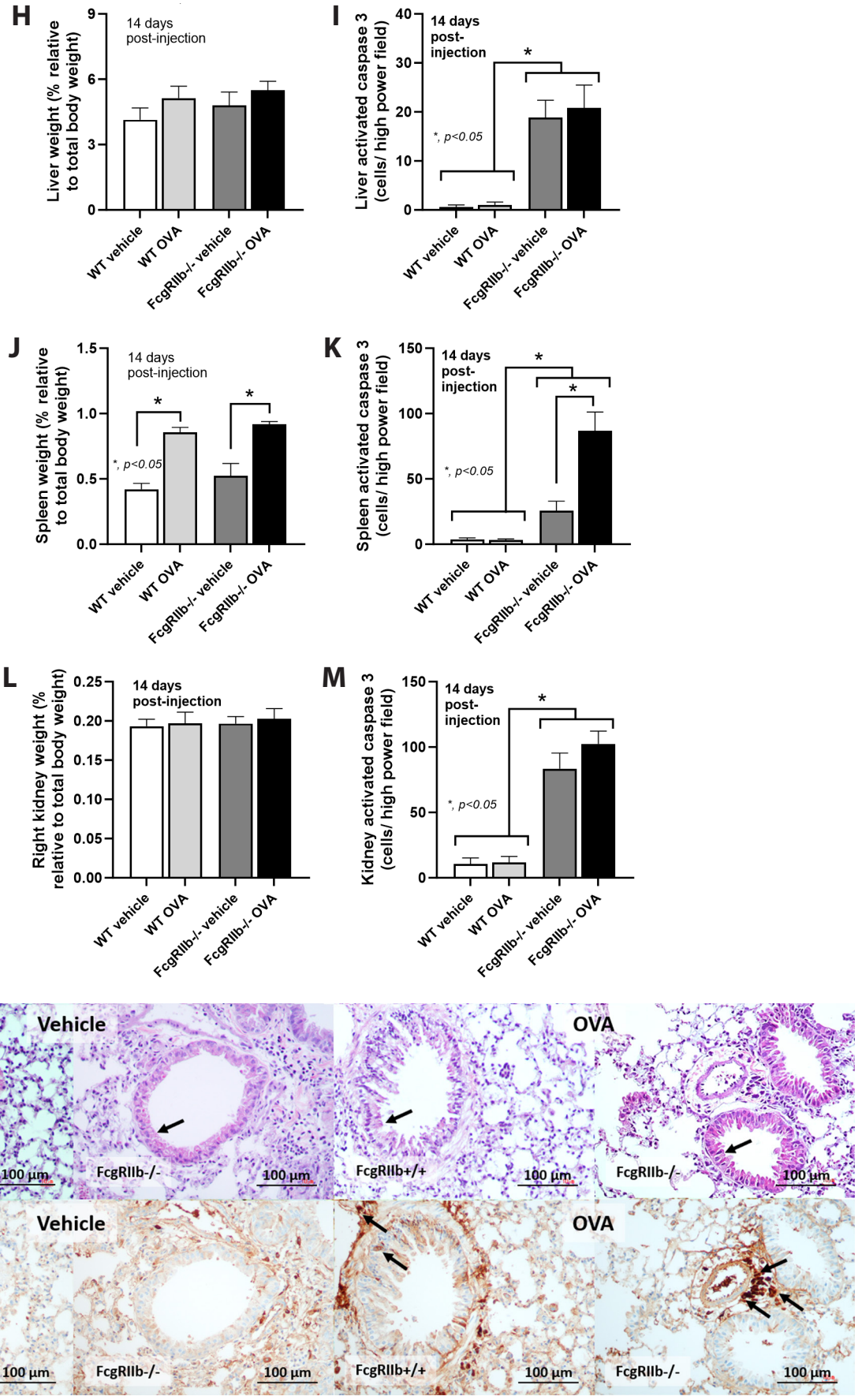

Figure 1. (Continued)

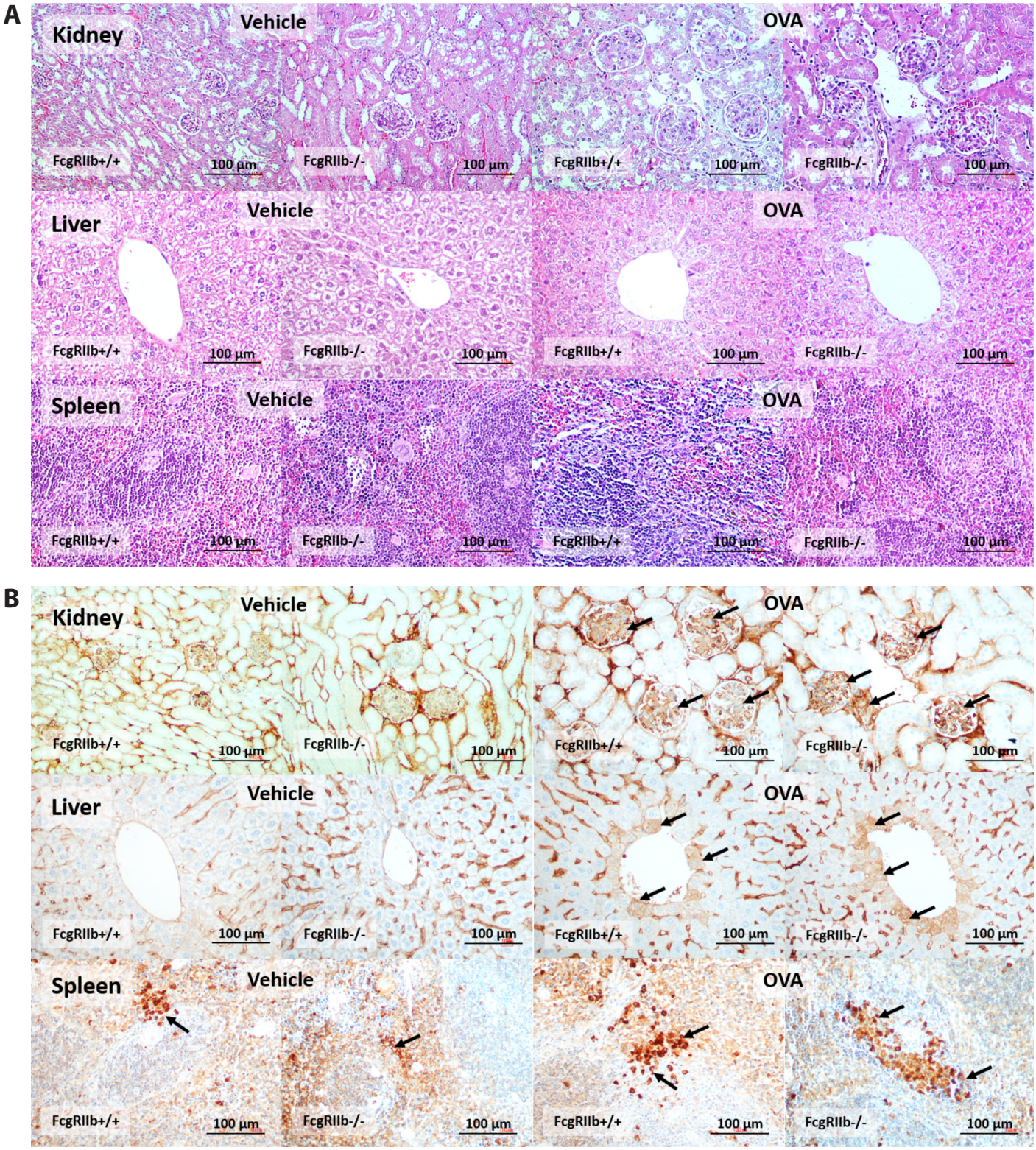

Figure 2. The representative histological pictures of mice with ovalbumin (OVA) or vehicle administration in several organs (kidney, liver, and spleen) as stained by Hematoxylin & Eosin (H&E) staining (A) and activated caspase 3 immunohistochemistry (B) are demonstrated. The arrows indicate examples of activated caspase 3 positive cells (brown colour cells).

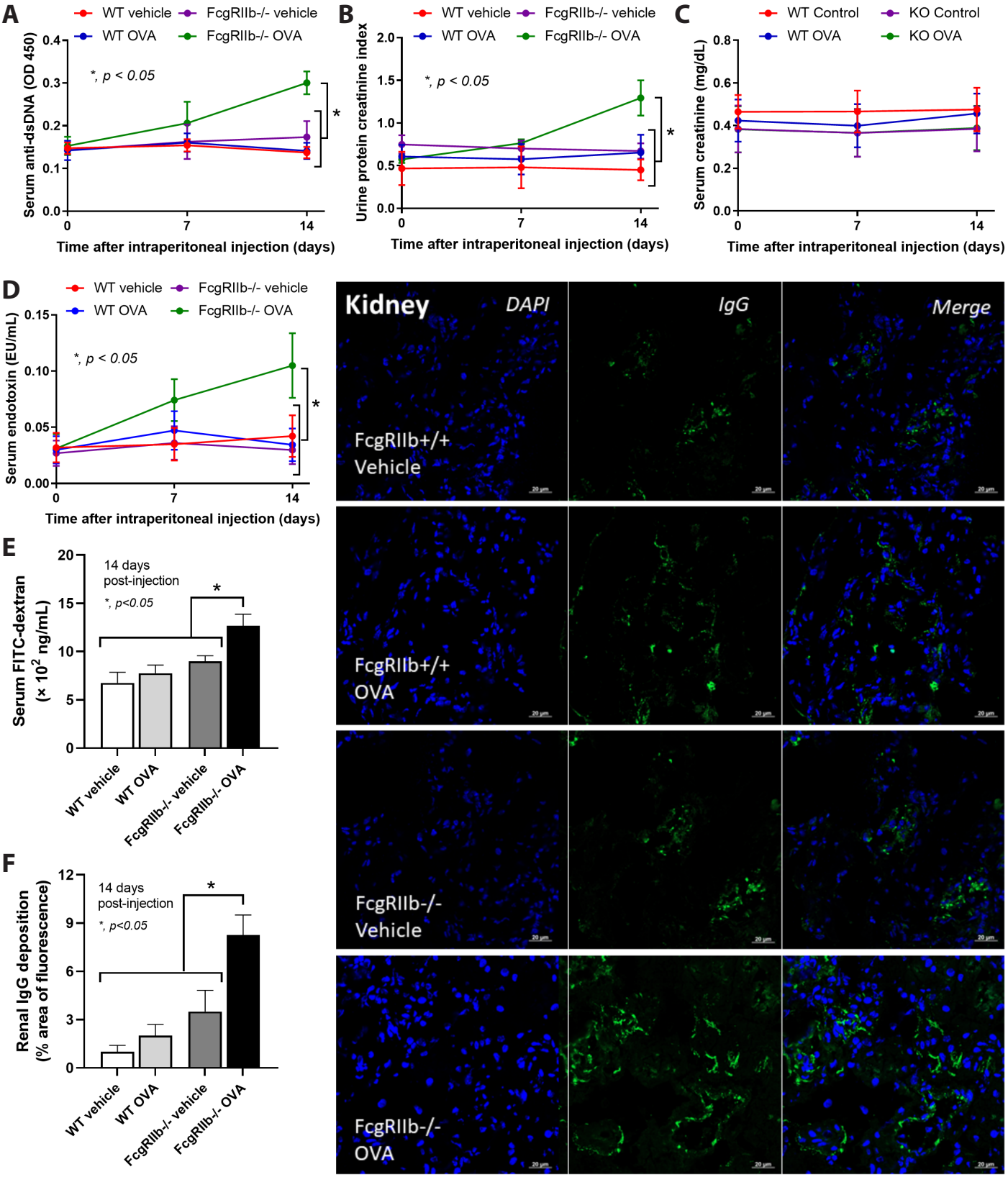

Figure 3. Characteristics of wildtype (WT) and FcgRIIb-/- mice with ovalbumin (OVA) or vehicle administration as indicated by the time-point of serum anti-dsDNA, urine protein creatinine index, serum creatinine, and serum endotoxin (A-D) and the parameters at 14-day post-injection as determined by gut permeability defect (FitC-dextran assay) (E), and IgG deposition in kidneys with the representative immunofluorescent pictures (F), are demonstrated. (n = 5 - 7/group). *, $p < 0.05$ between the indicated groups

Lupus exacerbation and leaky gut in ovalbumin (OVA)-administered FcgRIIb-/- mice through the enhanced renal immune complex deposition and altered dendritic cell functions

Due to the age-related lupus characteristics in FcgRIIb-/- mice, the onset of lupus nephritis (proteinuria with elevated anti-dsDNA) is usually as early as 16 weeks old.[13,22,36] Surprisingly, early lupus nephritis (increased serum dsDNA and proteinuria, but not elevated serum creatinine) was demonstrated in 8-week-old FcgRIIb-/- mice just after 2 weeks of OVA administration (10-week-old mice) (**Figure 3A-C**). Because of the possible correlation between lupus activities and endotoxemia from gut translocation (leaky gut) due to immune complex deposition in the gut,[14,26] serum endotoxin and immune complex deposition were examined. Indeed, only OVA-administered FcgRIIb-/- mice, but not other groups, demonstrated leaky gut (endotoxemia and FitC-dextran assay) together with renal immune complex deposition (immunofluorescence of immunoglobulin G staining) (**Figure 3D-F**). These data indicate a possible crosstalk between IgE immunoglobulin isotype class switching which is depending on T helper 2 (Th2), and interferon-gamma (IFN-γ)-mediated IgG production from Th1in FcgRIIb-/- lupus-prone mice.[37] Because there are Fc gamma receptors (FcgRs) on myeloid cells, such as macrophages, neutrophils, and dendritic cells (DC), but not on T cells,[5,8,11,12] the property of FcgRIIb-/- DCs might different from the WT cells. Then, DCs were derived from mouse bone marrows and activated by lipopolysaccharide (LPS) or OVA (alone or in combination) before determining the expression of the major histocompatibility complex class II (MHC II) and CD80.

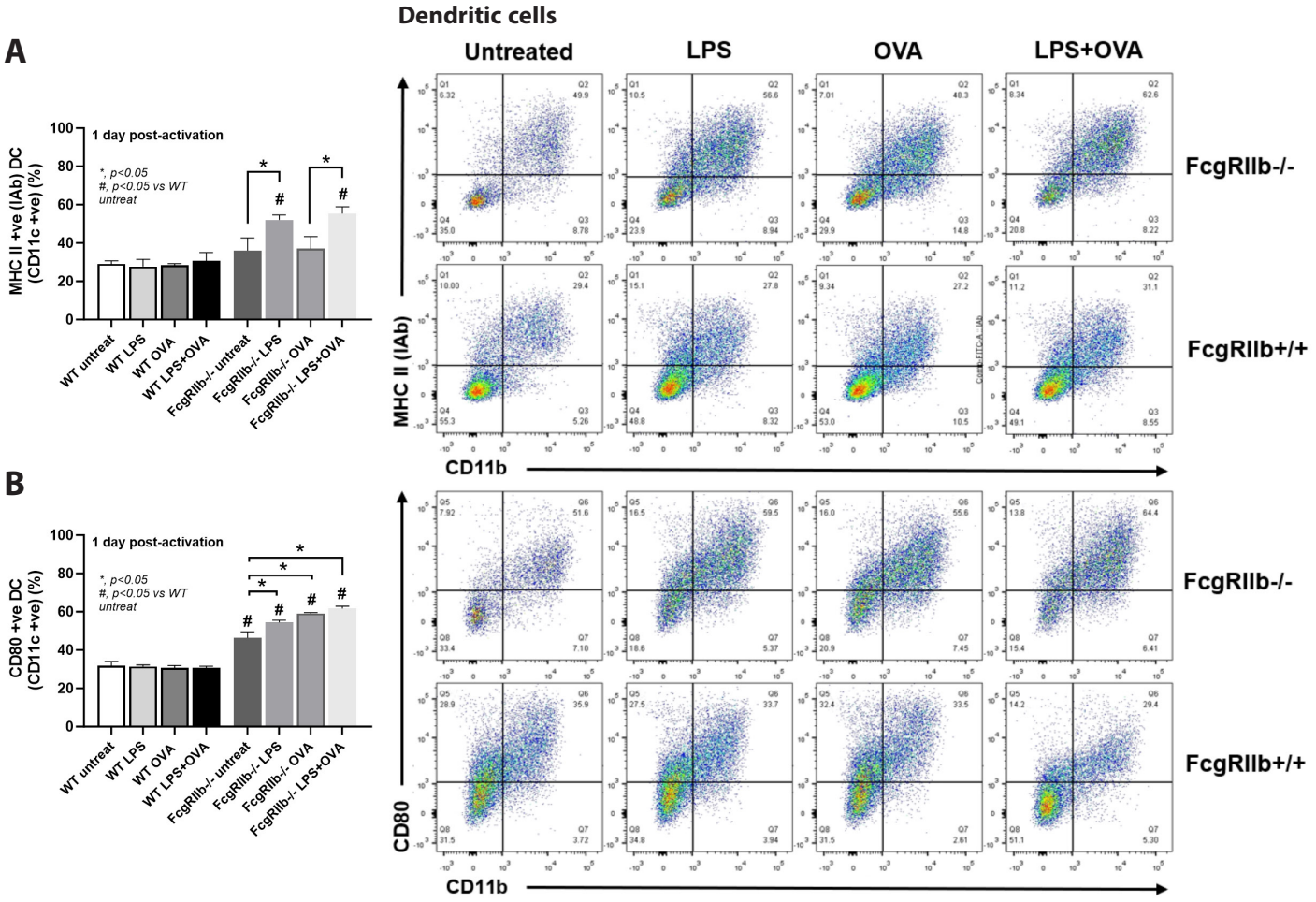

Figure 4. Characteristics of bone marrow-derived dendritic cells (DCs) from wildtype (WT) and FcgRIIb-/- mice after 1-day activation by culture media (untreated), ovalbumin (OVA), lipopolysaccharide (LPS), or combined LPS and OVA (LPS+OVA) as indicated by flowcytometry analysis with representative pictures of activated DCs using major histocompatibility complex II (MHC II) or CD80 with CD11c (A, B) are demonstrated. Expression of interferon-gamma (IFN-γ) on T cells from WT mice after the 3-day co-culture with the activated DCs (untreated, OVA, LPS, and LPS+OVA) from WT and FcgRIIb-/- mice (C) are also indicated. The results were derived from isolated triplicated experiments. *, $p < 0.05$ between the indicated groups

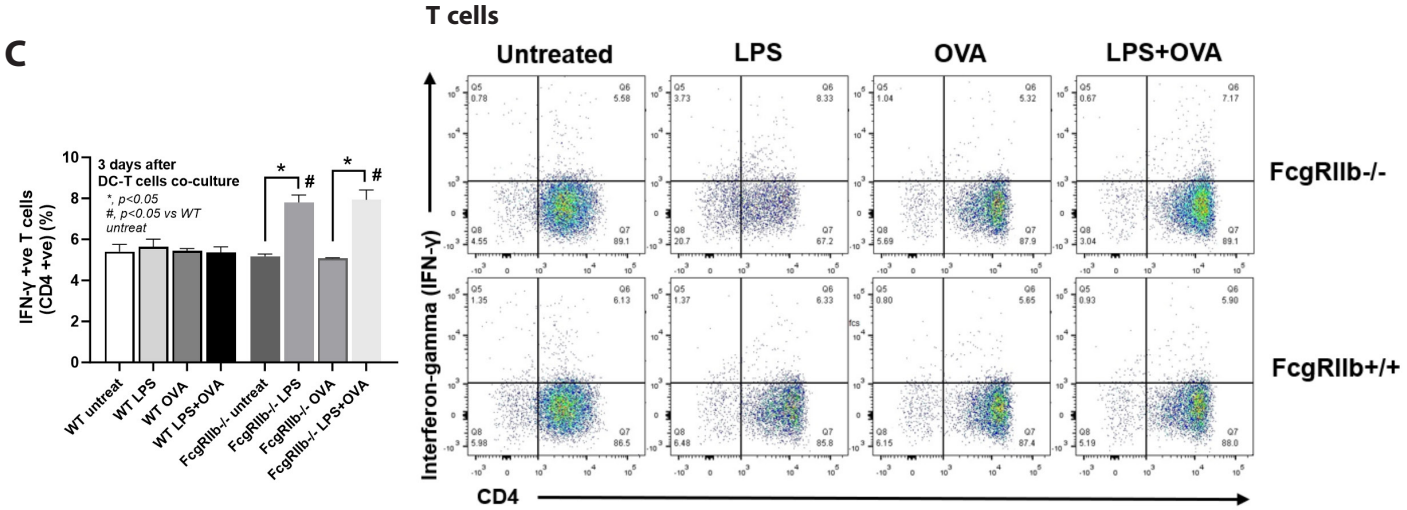

Figure 4. (Continued)

As such, the activation could not alter MHC II and CD80 in wildtype DCs (**Figure 4A, B**), while all conditions elevated CD80 in FcgRIIb-/- DC (**Figure 4B**). In parallel, only LPS and LPS+OVA (but not OVA alone) induced MHC II in FcgRIIb-/- DC (**Figure 4A**). Also, the co-culture between wildtype T cells with LPS- or LPS+OVA-administered FcgRIIb-/- DCs surprisingly elevated IFN-γ (**Figure 4C**). There was no increased IFN-γ after the co-incubation between wildtype T cells with wildtype DCs (all conditions) or OVA-administered FcgRIIb-/- DCs (**Figure 4C**). Although there was no difference between activation by LPS alone and LPS plus OVA in both WT and FcgRIIb-/- DCs indicating the major impact of LPS than OVA, the influence of OVA was demonstrated by the higher CD80 on OVA-activated FcgRIIb-/- DCs compared with control FcgRIIb-/- DCs (**Figure 4B**). Perhaps, the selected concentration of LPS could not activate wildtype DCs resulting in the null effect on IFN-γ production after the co-culture of T cells with LPS-activated wildtype DCs (**Figure 2B**). These *in vitro* data indicated the possible more prominent antigen processing property of FcgRIIb-/- DCs that subsequently induced profound IFN-γ production from T cells compared with wildtype DCs.

Discussion

Allergy and lupus exacerbation

Allergy, a common disease worldwide, is an immune hypersensitivity against several substances in the environment which mostly presents as allergic rhinitis, asthma, and dermatitis,[38] referred to as "history of atopy", that is based on the hypersensitivity type I (histamine-associated conditions due to the Th2 immunoglobulin isotype class switching from IgG to IgE)[39] and type IV (IFN-gamma-producing CD4+ T cell-mediated diseases).[40] On the other hand, lupus pathogenesis is correlated with hypersensitivity type II (recognition of self-antigens through auto-antibodies) and type III (immune complex-mediated diseases).[41] Despite different pathogeneses between allergy and lupus, allergic disorders are commonly found in patients with lupus, and allergies to some drugs occasionally be related to lupus flares.[38] Although both asthma and allergic rhinitis models can be used as Th2-associated mouse models, only asthma model is selected here due to our laboratory limitation.

Using 8-week-old FcgRIIb-/- asymptomatic lupus mice, OVA induced similar Th2-based responses in the wildtype mice as indicated by serum IgE and IL-4 together with lung histopathology. For the systemic impact, FcgRIIb-/- mice demonstrated spontaneous apoptosis in livers and kidneys supported the lupus characteristics.[1] With OVA activation, the elevated splenic apoptosis was found only in OVA-administered FcgRIIb-/- mice, perhaps correlated with the different immune responses due to the loss of inhibitory FcgRIIb signaling.[1] Surprisingly, OVA activation exacerbates early lupus nephritis (increased anti-dsDNA and proteinuria) in 10-week-old FcgRIIb-/- mice (2 weeks after OVA administration) which normally demonstrated at 16 weeks old mice.[1] In parallel, gut permeability defect (FitC dextran assay) and endotoxemia were also detected in OVA-activated FcgRIIb-/- mice, perhaps due to the increased deposition of the immune complex in the intestines.[6-8] The increased immune complex deposition in the kidney after OVA stimulation in FcgRIIb-/- mice, but not in wildtype, implying the elevated auto-antibody production with enhanced immune complex after OVA-induced allergy only in FcgRIIb-/- lupus-prone mice. Although FcgRs are found on myeloid immune cells and B cells, not T cells, FcgRs in DCs might determine the response directions of Th cells.[42,43] Because endotoxemia is another factor that possibly correlates with lupus exacerbation,[26] LPS might be another factor that activates DCs in OVA-administered FcgRIIb-/- mice. With the selected concentration, LPS and OVA could not induce wildtype DCs. In contrast, LPS enhanced both MCH II and CD80 in FcgRIIb-/- DCs, while OVA elevated only CD80 in FcgRIIb-/- DCs. The active DCs from LPS or LPS+OVA induction elevated IFN-γ, while CD80+ DCs from OVA alone could not increase IFN-γ expression. Hence, there was no crosstalk of IFN-γ production (a Th1 response) after OVA induction (Th2 activation) in FcgRIIb-/- DCs. The lupus exacerbation in OVA-administered FcgRIIb-/- mice might be due to the increased abundance of active CD80+ DCs that activate Th cells in a pro-inflammatory direction.

Although OVA-administered FcgRIIb-/- DCs induced similar level of IFN-γ production by T cells compared with control DCs (**Figure 4C**), OVA increased the abundance of activated DCs (CD80+ cells) that might exacerbate lupus activity. Thus, our results supported a possible lupus exacerbation after allergic reactions in FcgRIIb-/- mice, partly through the pro-inflammatory DCs due to the loss of the inhibitory FcgRIIb receptor. Hence, the OVA-induced inflammation might induce auto-antibody and immune complex deposition in FcgRIIb-/- mice as indicated by increased serum dsDNA, IgG staining in kidneys, and leaky gut. Notably, immune complex deposition in the gastrointestinal tract in patients with lupus is well-known even without intestinal symptoms.[44-46] Moreover, lupus exacerbation might also be worsened by leaky gut-induced endotoxemia as LPS more prominently activated FcgRIIb-/- DCs than wildtype cells through the induction of both MHC II and CD80 on DCs. Thus, OVA might be a weak exacerbating factor of lupus but endotoxemia from immune complex deposition in the intestines during active lupus might strongly induce lupus characteristics in FcgRIIb-/- mice. Although more studies are needed, our pilot results indicate a possible clinical perspective that the lupus flare might be a result of the histamine-mediated allergy and anti-histamine might be useful.

There were several limitations in our current manuscript. First, the OVA-induced asthma model was not the only Th2-mediated model, and the use of other models; for example, the allergic rhinitis model, might result in a more solid conclusion. Second, the discovery from animal models always needs validation in the real human situation and/or in patients. Third, LPS could not activate WT DCs here which might be due to the inappropriate concentration or preparation. The different adjustments of LPS in vitro might lead to different conclusions.

In conclusion, exacerbation of lupus nephritis with endotoxemia from the leaky gut in asymptomatic lupus-prone FcgRIIb-/- mice was demonstrated through OVA induction. While OVA similarly induced allergic responses in both wildtype and FcgRIIb-/- mice, FcgRIIb-/- DCs were more susceptible to the activations than wildtype DCs causing more profound inflammation in FcgRIIb-/- mice. For the clinical translation, the control of allergic conditions might be beneficial for patients with lupus, more studies are warranted.

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Author Contributions

- Conceptualization, A.L.
- Methodology, T.B., K.H., J.C. and A.L.
- Software, T.B., W.S. and A.L.
- Validation T.B., W.S. and A.L.
- Formal analysis, W.S. and A.L.
- Investigation, T.B. and W.S.
- Resources, T.B., P.R. and A.L.
- Data curation, T.B. and A.L.
- Writing-original draft preparation, T.B. and A.L.
- Writing-review and editing, T.B. and A.L.
- Visualization, T.B. and A.L.
- Supervision, T.B. and A.L.
- Project administration, T.B., J.M. and A.L.
- Funding acquisition, P.R. and A.L.
- All authors have read and agreed to the published version of the manuscript

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Institutional Review Board Statement

The study was conducted according to the approved animal study protocol by the Institutional Animal Care and Use Committee of the Faculty of Medicine, Chulalongkorn University (ASP SST 12/2562).

Informed Consent Statement

Not applicable.

Data Availability Statement

The data is contained within the article.

Conflicts of Interest

The authors declare no conflict of interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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