

Treatment with IMO-3100, a novel TLR7 and TLR9 dual antagonist, inhibits disease development in lupus prone NZBW/F1 mice

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INTRODUCTION

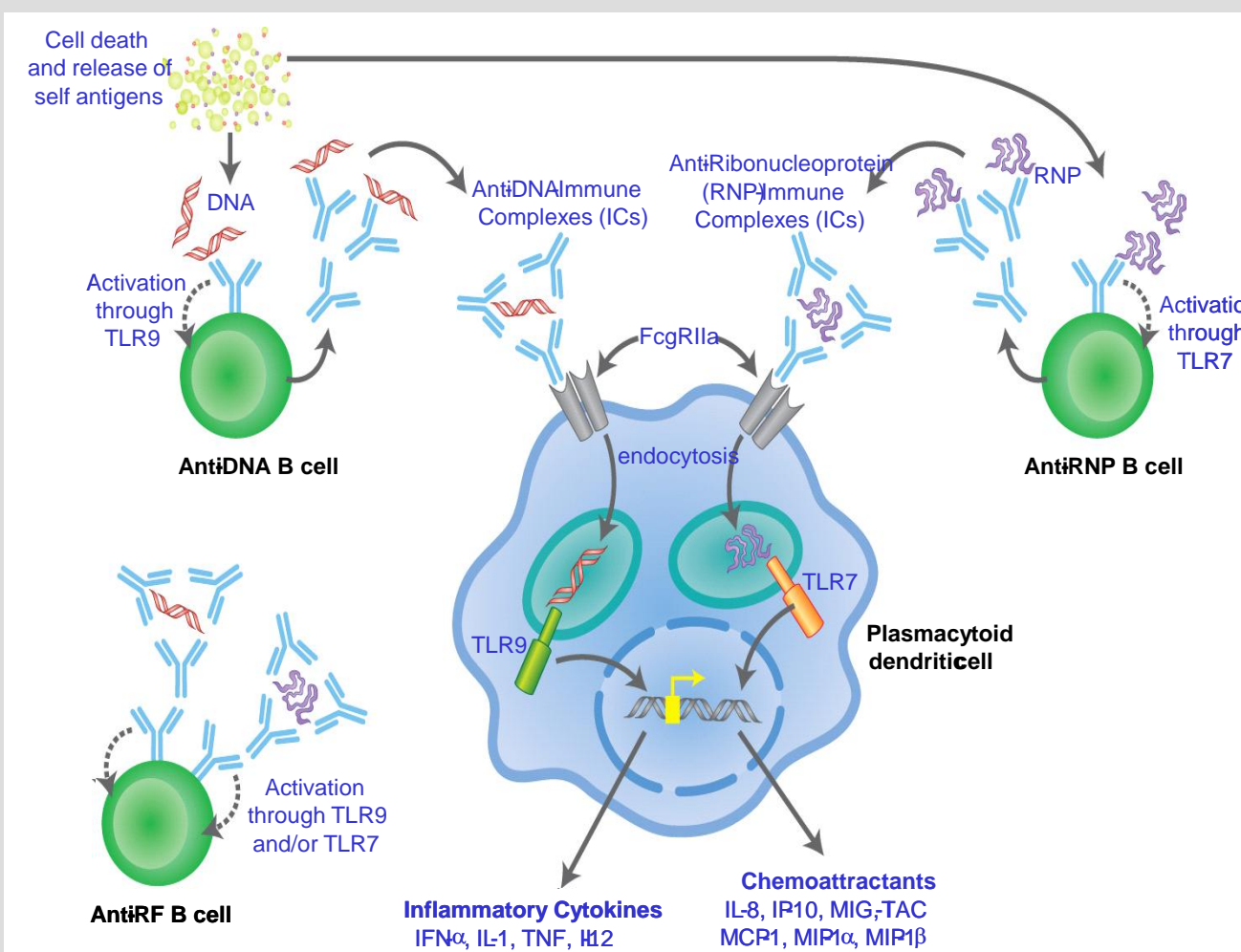
Toll-like receptors (TLR) play a key role in pathogen recognition and activation of innate immunity. Of the ten TLRs identified in humans, TLR7 and 8 recognize single-stranded viral RNA, and TLR 9 recognizes unmethylated CpG dinucleotide-containing bacterial and viral DNA.

TLR7 and 9 play a central role in maintenance and progression of systemic lupus erythematosus (SLE) in humans and in mouse models. Engagement of TLR9 with CpG-motif-containing DNA can contribute to the production of pathogenic anti-DNA autoantibody in vitro and in vivo (1-3). Lupus patients have increased gene expression of TLR 7 and 9 (4), and increased TLR9-positive B cells in the peripheral blood mononuclear cells (5). TLR7 plays a critical role in acceleration of systemic autoimmunity in murine lupus. The Tlr7 gene duplication resulting from the translocation of the X chromosome to the Y chromosome has been shown to contribute to the acceleration of SLE in the Yaa-bearing mice (6, 7). Similarly, deletion of TLR8 is associated with TLR7 hyperresponsiveness and development of lupus like syndrome (8).

Through extensive structure-activity relationship studies, we have identified a novel class of DNA-based compounds that act as dual antagonists of TLR7 and TLR9 by blocking immune responses mediated through these receptors (9-11). In preclinical studies, our TLR7 and 9 antagonist candidates have shown potent activity in disease models of rheumatoid arthritis, lupus, multiple sclerosis, psoriasis and atherosclerosis (12-16).

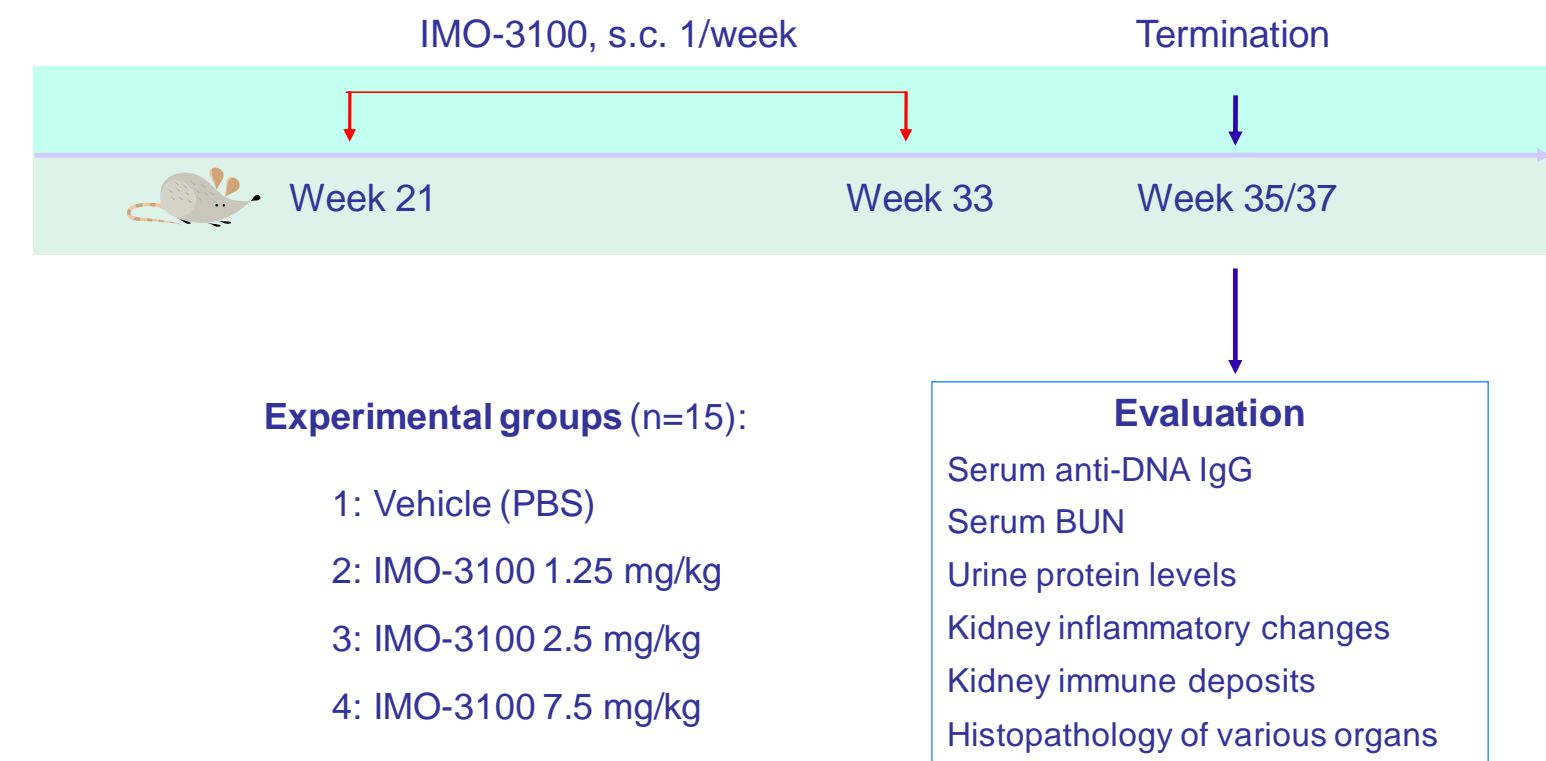
In the present study, we have examined the therapeutic efficacy of IMO-3100, a dual TLR7/9 antagonist, in lupus prone NZBW/F1 mice.

Immune Complex/Apoptotic Cell Debris and TLRs

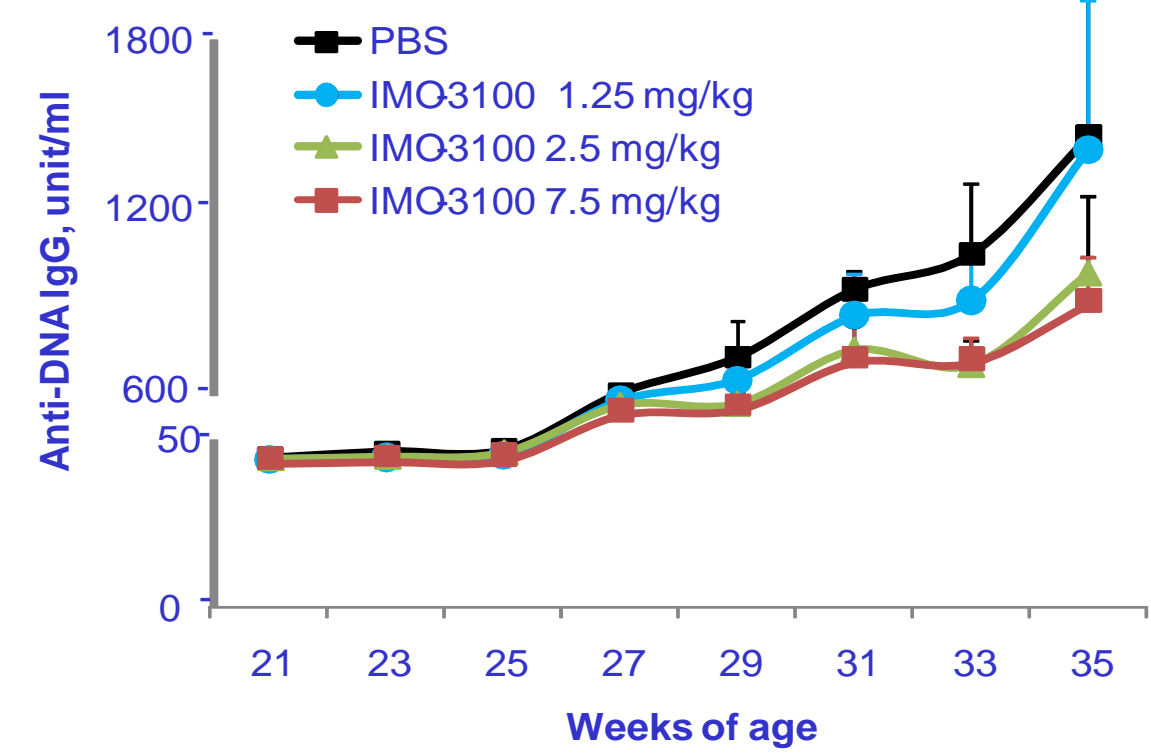


EXPERIMENTAL DESIGN & RESULTS

Protocol for Evaluation of IMO-3100 in Lupus Prone NZBW/F1 Mice

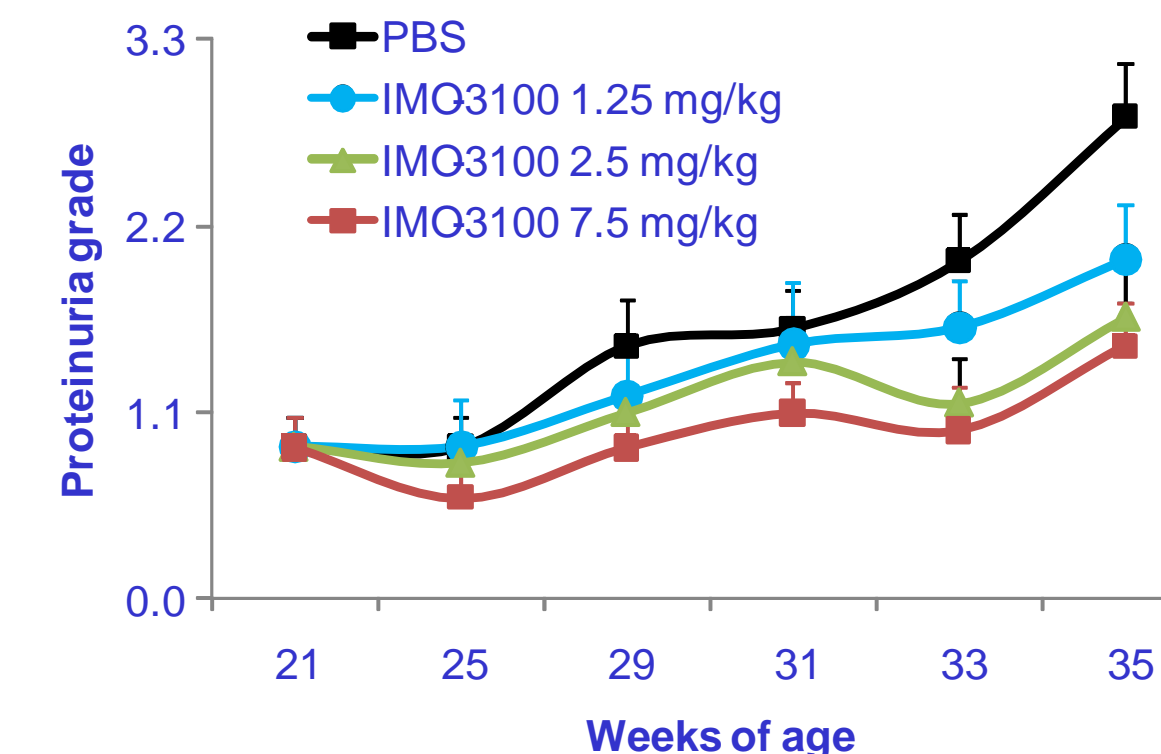


IMO-3100 Decreases Serum Anti-DNA IgG Levels



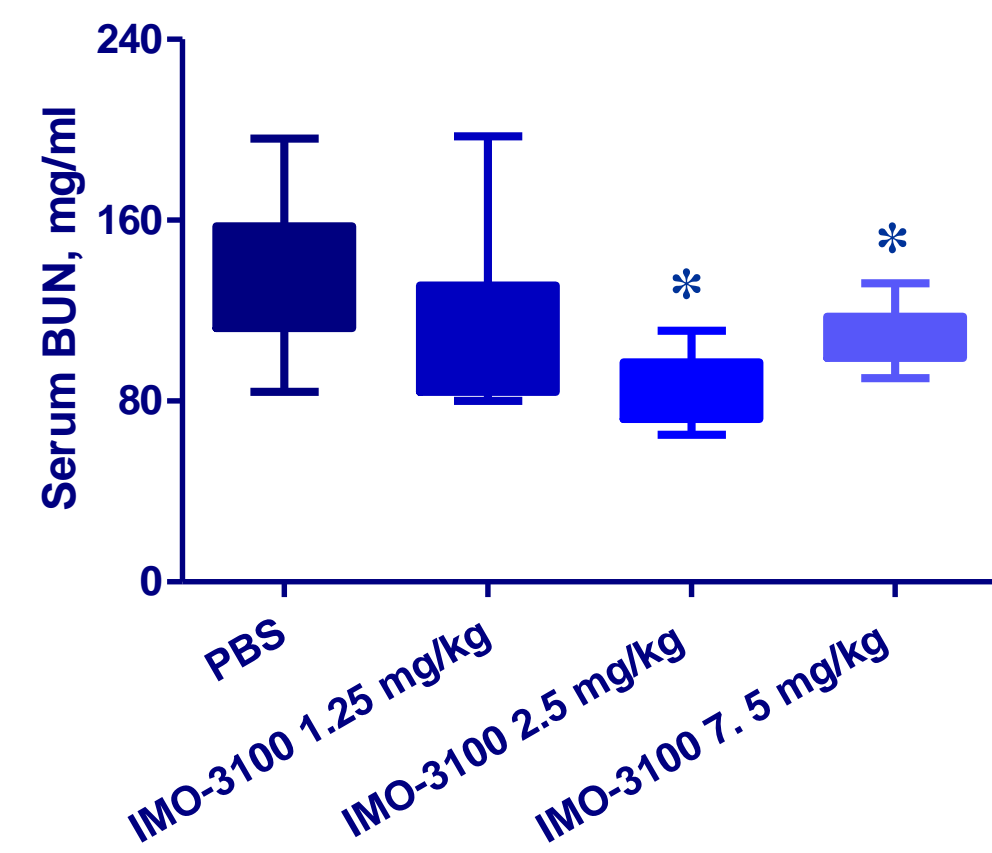
IMO-3100 at 2.5 and 7.5 mg/kg/week, significantly decreased serum anti-DNA IgG levels from week 27 to 35 (p < 0.05)

IMO-3100 Suppresses Urine Protein Levels



IMO-3100 at 2.5 and 7.5 mg/kg/week, significantly decreased serum anti-DNA IgG levels from week 29 to 35 (p < 0.05). Grade of urine protein levels with the Multistix test strips (Bayer): 0: no proteinuria; 1: ≥ 30 mg/dl; 2: ≥ 100 mg/dl; 3: ≥ 300 mg/dl; 4: ≥ 2000 mg/dl

IMO-3100 Suppresses Serum Blood Urea Nitrogen (BUN) Levels



* P < 0.05 compared with PBS group

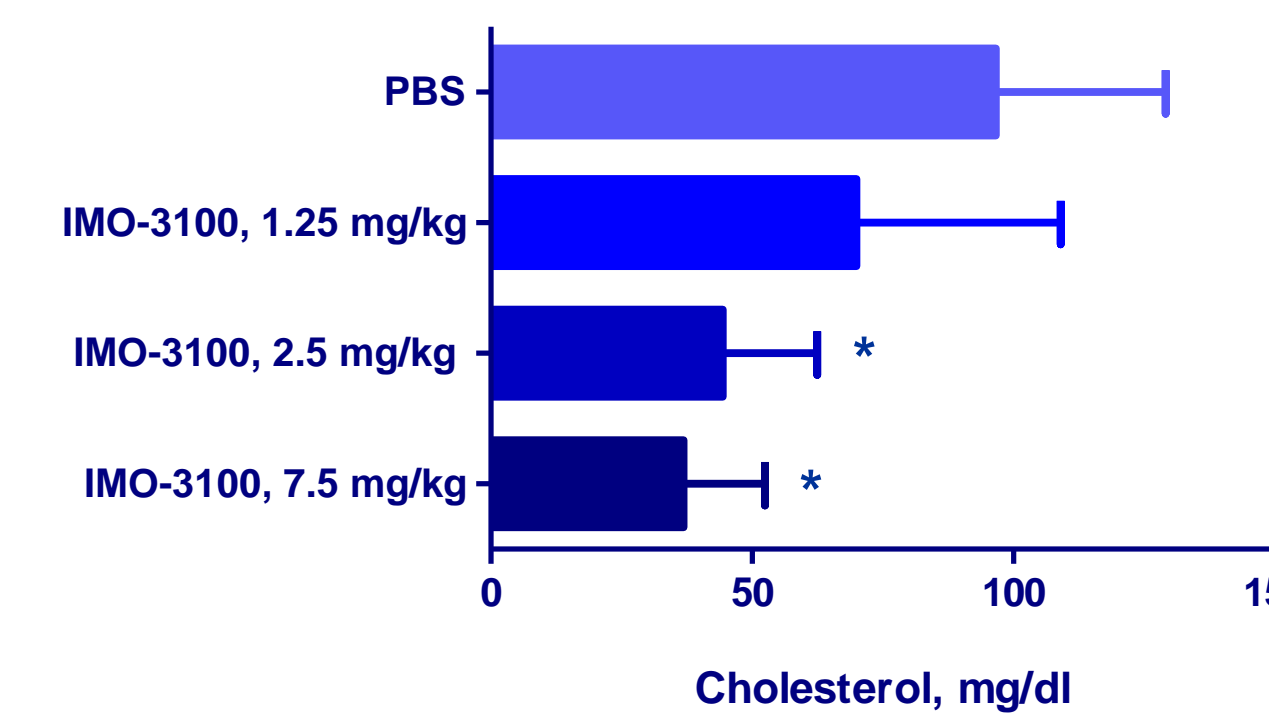
IMO-3100 Reduces Histopathological Changes in Kidney, Liver, Spleen, Salivary Gland and Lymph Nodes

Experimental groups →	PBS	IMO-3100
Kidney		
Glomerulopathy	2.6	1.6
Lymphocyte infiltrates	2.4	1.3
Tubule changes	2.0	0.0
Spleen		
Lymphoid hyperplasia	2.6	2.2
Extramedullary hematopoiesis	2.5	0.0
Lymph Nodes		
Lymphoid hyperplasia	3.4	0.3
Salivary Glands		
Lymphocyte infiltrates	2.5	2.6
Liver		
Fatty Change	0.2	0.0
Hydropic degeneration	0.4	0.0
Extramedullary hematopoiesis	0.0	1.3

PBS and 7.5 mg/kg Groups at week 37

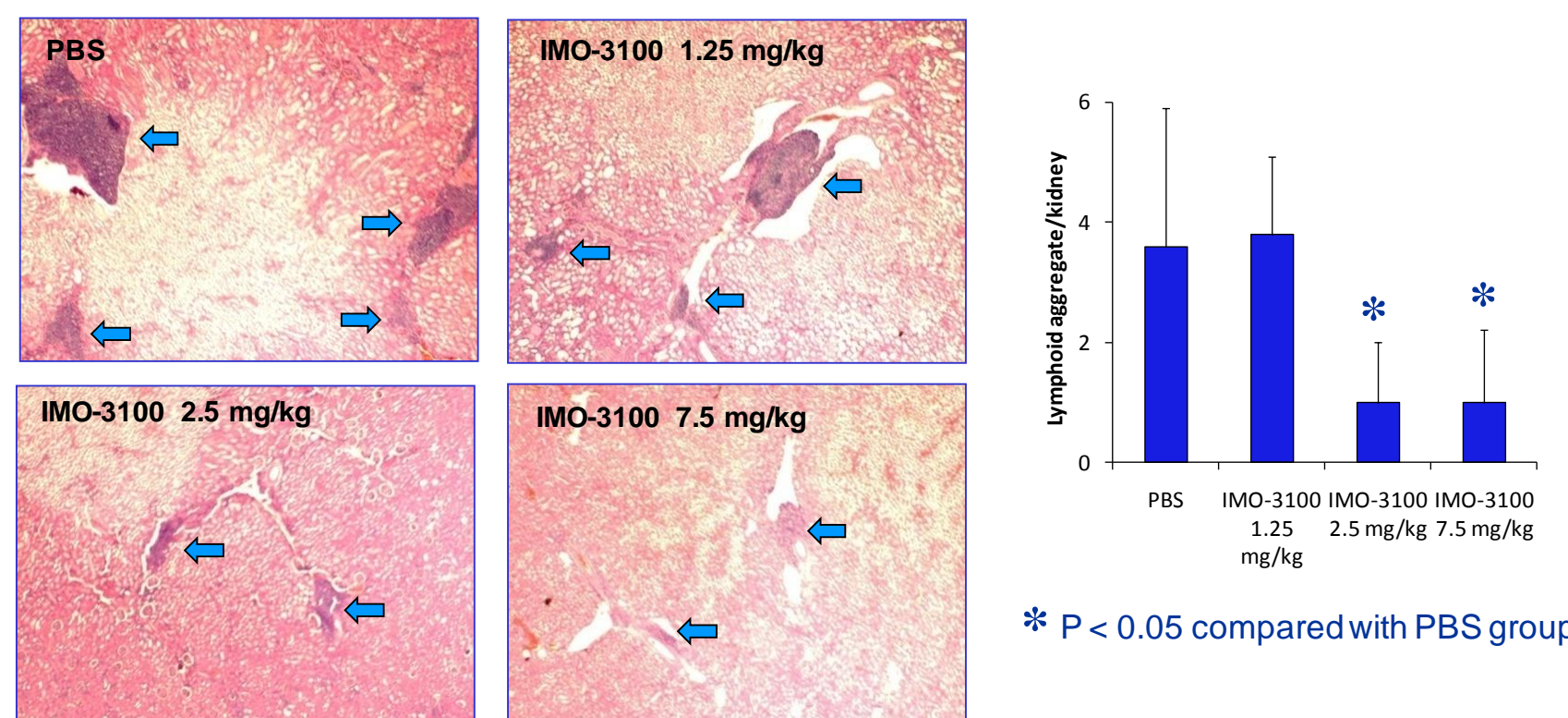
Tissues from multiple mice (N=5) were microscopically examined and the histological findings were scored as: 0= within the range of normal; 1=slight or minimal findings, 2=mild, 3=moderate, 4=marked, 5=severe abnormal.

IMO-3100 Suppresses Serum Cholesterol Levels



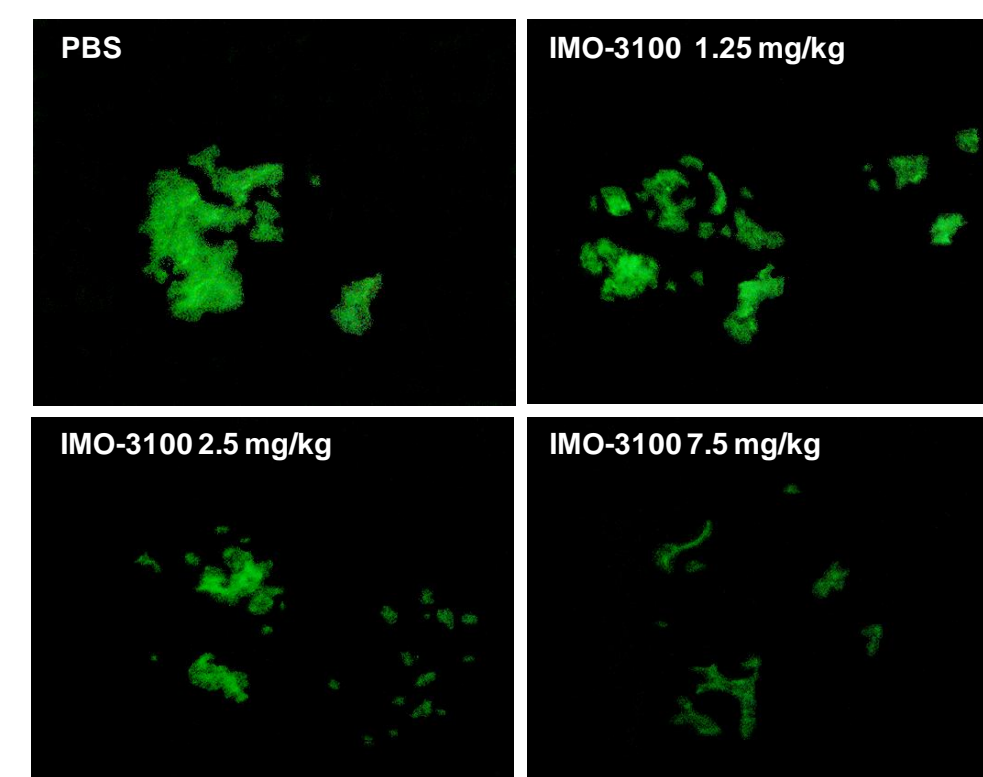
* P < 0.05 compared with PBS group

IMO-3100 Reduces Inflammatory Cell Infiltration in the Kidneys



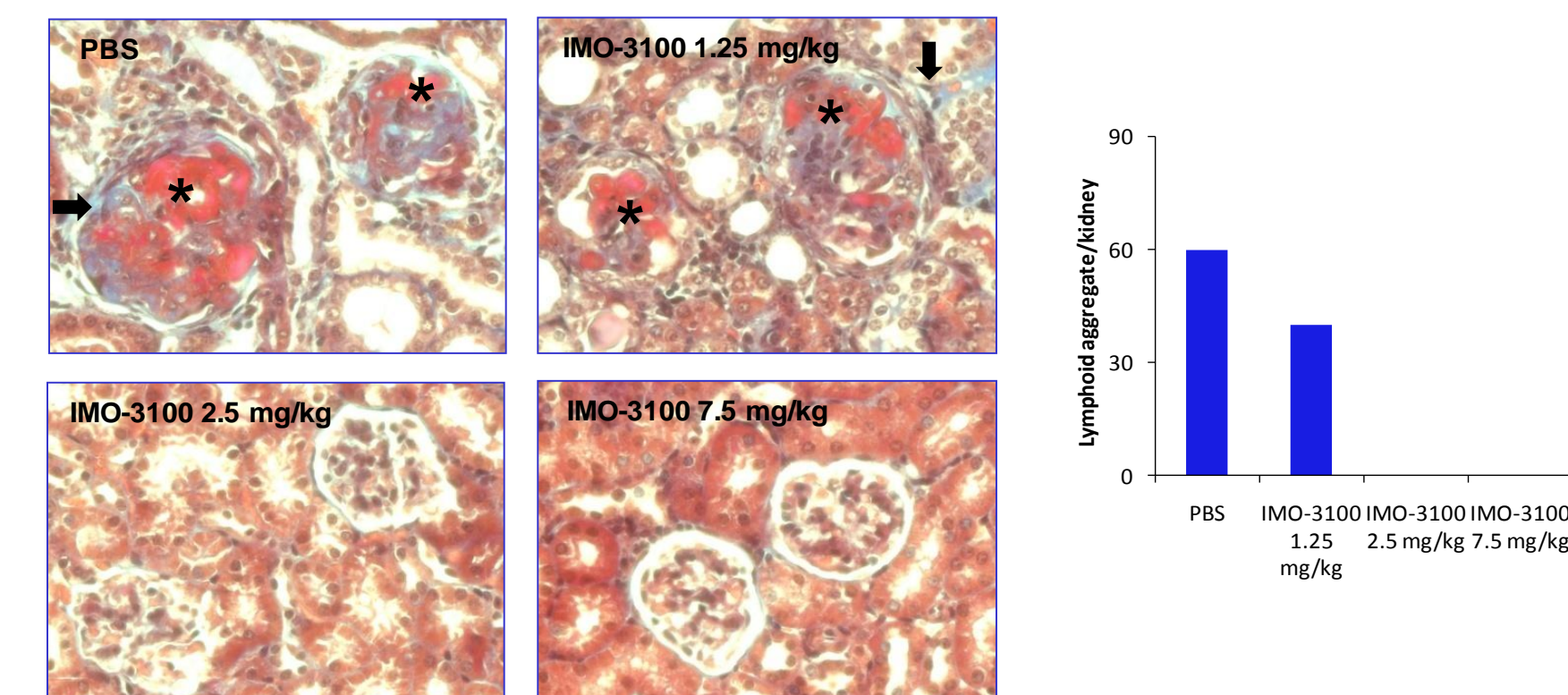
Renal interstitial inflammation with the formation of lymphoid aggregates (blue arrows) at age 35 weeks are less abundant in the kidneys from mice treated with IMO-3100 at 2.5 and 7.5 mg/kg/week (HE staining, magnification x 20).

IMO-3100 Reduces Glomerular Immune Complex Deposits in the Kidneys



Extensive glomerular immune complex deposits (green color) are present in the kidneys from PBS-treated mice at week 35, but are remarkably decreased in mice treated with IMO-3100 at 2.5 and 7.5 mg/kg/week (IgG Immunofluorescence staining, x 200)

IMO-3100 Reduces Glomerular Necrosis and Fibrosis in the Kidneys



Renal glomerular sclerosis, characterized by necrosis (stained red; asterisk) and fibrosis (stained blue; arrows) at age 35 weeks are less abundant in the kidneys from mice treated with IMO-3100 at 2.5 and 7.5 mg/kg/week (Masson-Trichrome stain x400)

SUMMARY

- IMO-3100, a dual TLR7 and TLR9 antagonist, exerts therapeutic effect in NZBW/F1 mice
 - Lowers serum anti-DNA IgG levels
 - Reduces blood urea nitrogen (BUN) levels
 - Suppresses urine protein levels
 - Has protective effect on histological alterations in a number of organs
 - Reduces kidney leukocyte infiltration, necrosis and fibrosis
 - Reduces glomerular immune complex deposit
 - Corrects lupus associated dyslipidemia by reducing serum cholesterol levels
- Data suggest that blocking of TLR7 and 9 is beneficial to the murine model of lupus.

• Results are in agreement with our previous preclinical data demonstrating that administration of IMO-3100 results in inhibition of disease development in murine autoimmune disease models, e.g., rheumatoid arthritis, multiple sclerosis, psoriasis and lupus.

Acknowledgements

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