

ラット半月体形成性糸球体腎炎モデル作製の検討 Study on preparation of a rat crescentic glomerulonephritis model

○Toshinori Moritani, Akihiro Ishimoto, Hitomi Matsuda, Shoko Kosugi, Mayumi Murata, Ayahito Kimura
NISSEI BILIS Co., Ltd., Shiga Laboratory

Introduction

Crescentic glomerulonephritis (CGN) is one of the main diseases that progress to end-stage renal disease. CGN refers to rapidly progressive glomerulonephritis syndrome (RPGN), which includes anti-neutrophil cytoplasmic antibody (ANCA)-associated nephritis, anti-glomerular basement membrane antibody (GBM Ab) nephritis, and various other kidney diseases. The number of patients with RPGN is increasing year by year. We aimed to develop produce a rat model of CGN crescentic glomerulonephritis by administering human myeloperoxidase (hMPO) and anti-GBM Ab.

Methods

This study was conducted as approved by the Institutional Animal Experiment Committee of NISSEI BILIS Co., Ltd., Shiga Laboratory.

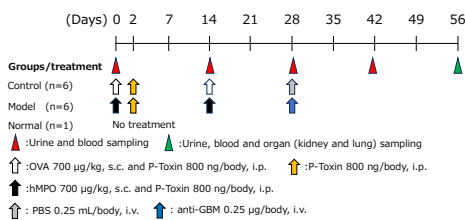
Materials and Methods

- Animals
Male WKY rats, 7 weeks old
(The Jackson Laboratory Japan, Inc.)
- Triggering substance
 - hMPO : human myeloperoxidase (Elastin Products)
 - OVA : ovalbumin (hMPO control, Chondrex)
 - CFA : complete Freund's adjuvant (Chondrex)
 - P-toxin : pertussis toxin (List Biological Laboratories)
 - anti-GBM Ab : affinity-purified rat monoclonal antibodies to $\alpha 4$ (IV) NC1 of rat glomerular basement membrane (Chondrex)
- Evaluation
 - Urinary parameters**
Urinary total protein (TP) and creatinine concentration were measured by 7180 clinical analyzer, Hitachi High-Tech Corp.
Hematuria (score 0-3), **proteinuria** (score 0-4), and **leucocyturia** (score 0-3) was scored by using urine test paper (Lifestix, Siemens Healthcare Diagnostics K.K.).
Urinary Albumin concentration was assayed by using ELISA kit (LBIS Rat Albumin ELISA Kit, FUJIFILM Wako Pure Chemical Corporation).
 - Serum parameters**
Serum Creatinine (S-Cre) and **CRP** concentration were measured by 7180 clinical analyzer, Hitachi High-Tech Corp.
Serum MPO-ANCA concentration was assayed by using ELISA kit (Rat MPO-ANCA ELISA Kit (HUAMEI BIOTECH Co., Ltd (Former Cusabio LLC)), code ; CSB-E08675r).
 - Histopathological examination**
HE, PAM, immunofluorescence (IF, stained for rat IgG), and **naphtol AS-D (ASD)** stain were performed.

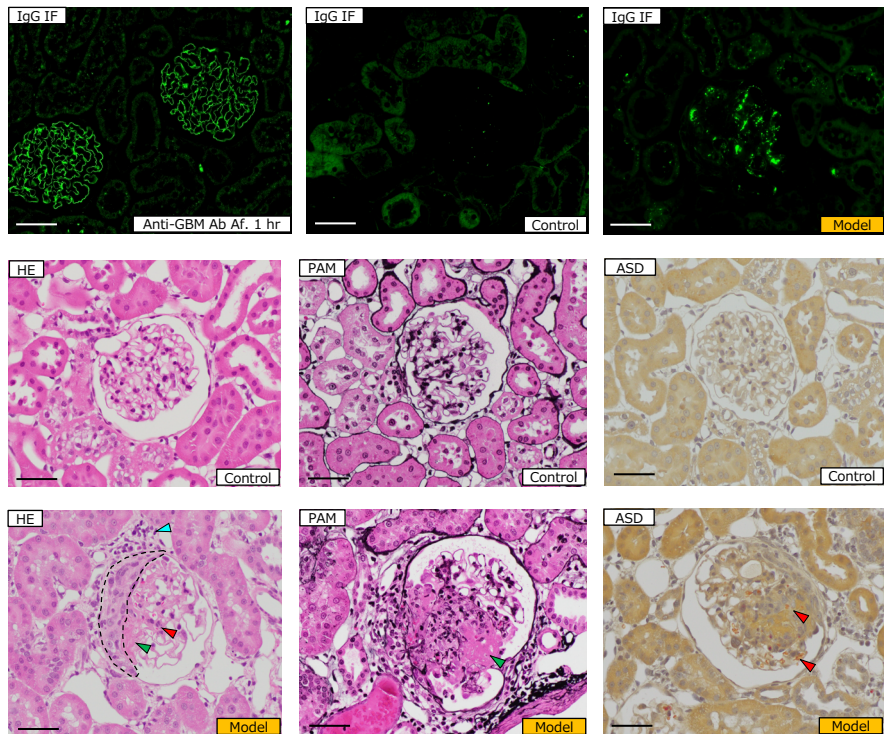
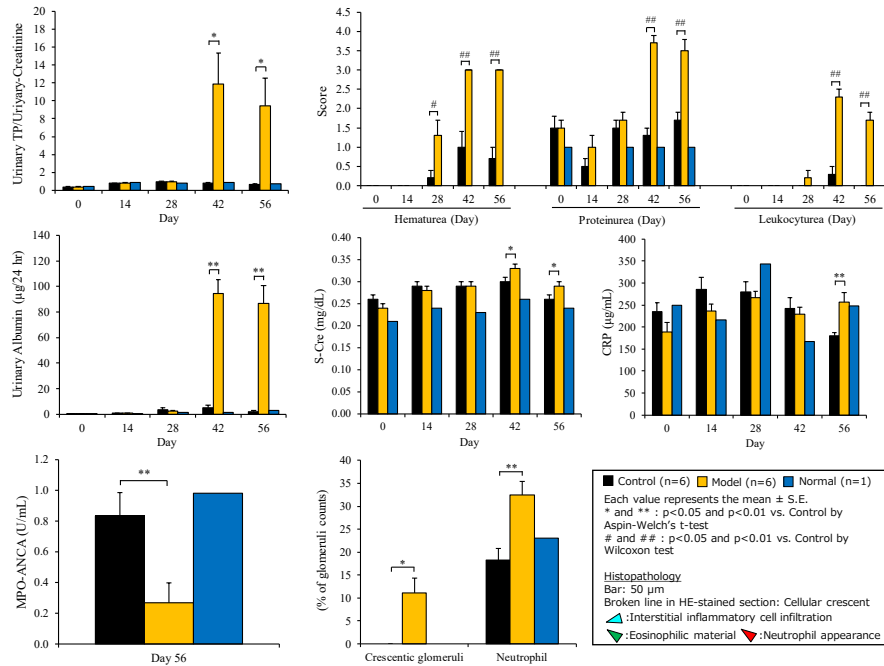
IgG IF:

FFPE sections were incubated with proteinase K for antigen retrieval and then stained for 60 minutes at room temperature with a 1:50 dilution of Alexa Fluor 488-conjugated goat anti-rat IgG (Abcam, code: ab150157).

Group design and study schedule



Results



Conclusion

Based on the urine, serum biochemical tests, and histopathological characteristics, a rat CGN model was successfully created. However, it did not match the main characteristics of major diseases, such as ANCA-associated vasculitis, which are prevalent in human CGN (RPGN).

Further detailed analysis will be conducted to clarify the features of this model.

	This model	Clinical Crescentic Glomerulonephritis		
Findings	Hematuria (+), proteinuria (+), S-Cre ↑, CRP ↑	Hematuria (+), proteinuria (+), S-Cre ↑, CRP ↑		
Disease type classification (Immunofluorescence)	Granular along the GBM	Pauci-Immune type	Linear type	Granular type
Serological marker	ANCA ↓	ANCA ↑	Anti-GBM Ab	Immune Complex (Anti-DNA Ab)
Major disease		ANCA-associated nephritis	Anti-GBM nephritis, Goodpasture Syndrome	IgA nephropathy, lupus nephritis