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PROBETEX
Experimental Pathology Resources™

Mesangioproliferative GN

Rat model

Induction

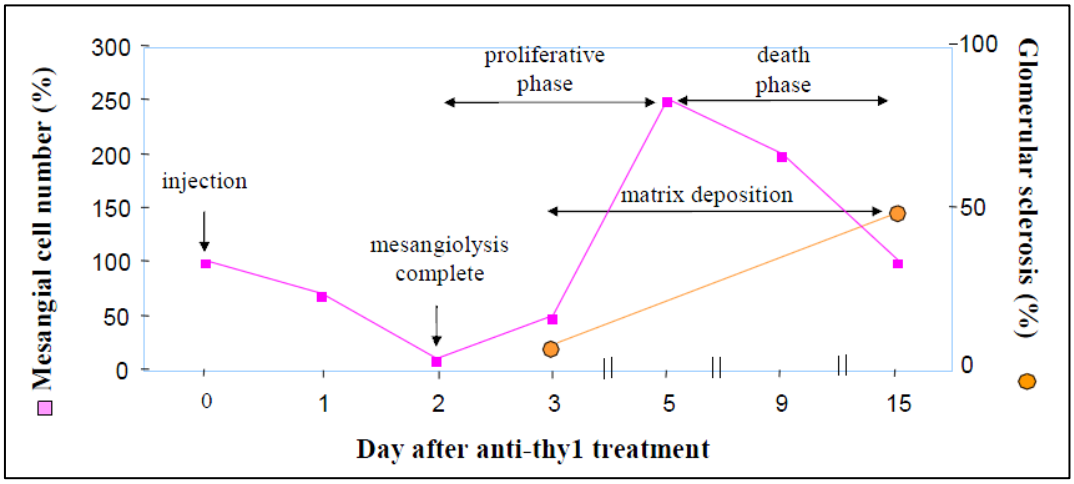
Sheep anti-thymocyte (ATS, Thy-1) serum, 0.5 ml/100gm body wt.

Mesangioproliferative glomerulonephritis:

Mesangial proliferation is a very common feature of many human glomerular diseases including IgA nephropathy, resolving post-infectious glomerulonephritis and a number of secondary glomerular diseases such as lupus nephritis, Schonlein-Henoch purpura, rheumatoid arthritis, liver cirrhosis, Alport's syndrome, and diabetic nephropathy (1,2). The disease is characterized by varying degrees of mesangial hypercellularity and mesangial matrix expansion. In progressive cases these cellular changes may lead to glomerular capillary narrowing, sclerosis and capsular adhesions as a result of injury by a variety of immunologic, toxic, metabolic, mechanical, and inflammatory mediators (1,2). Although several experimental models have been developed, the most widely used model for the study of mesangial proliferation has been the anti-thymocyte (anti-Thy-1) model (3,4). Antibody to thymocytes (ATS) is reactive to a surface Thy-1 antigen present on rat mesangial cells (3,4). Administration of ATS induces a complement-dependent mesangiolysis followed by a rapid mesangial proliferative glomerulonephritis that peaks within 5 days after injection, and then resolves over time (3,4). The disease does not accumulate IgA, rather is an IgG mediated progression.

Disease Progression

Immune-mediated mesangioproliferative glomerulonephritis is an acute model involving antibody-induced mesangiolysis (day 1) followed by inflammatory cell infiltration (Day 1-3), mesangial cell proliferation (day 2-5) and matrix accumulation (day 4-9). The lesions are focal and segmental. Proteinuria up to 50 mg/day by day 7. The disease is resolves 2-4 weeks after induction. Multiple injections of antisera result in protracted and progressive disease.



Chaudhuri A et al (with Probetex, Inc.: Molecular mechanisms underlying mesangioproliferative kidney damage in a rat anti-thy-1.1 model of glomeruonephritis: A gene expression analysis. J Am Soc Nephrol 15:435A, 2004.

Dosing Regimens (Examples)

- Begin dosing at around 1 to 2 days and continue until termination (Day 14).
- Route of administration: SC, PO, IP, IV

Clinical Assessment

Body weights are recorded daily; Urine samples are collected by metabolic cages for proteinuria measurement prior to termination. Urine protein is measured using BioRad reagent and reported as mg/24 hours. General health assessed daily.

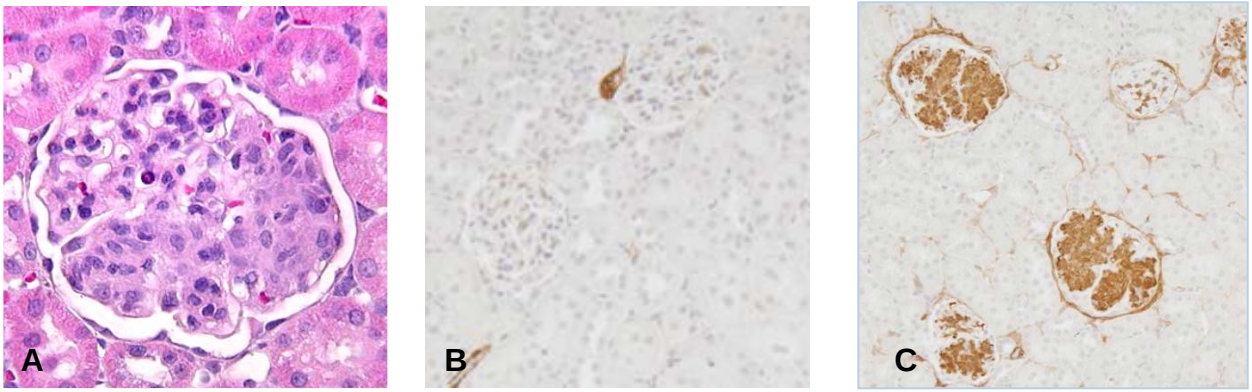
Histopathological Assessment

Kidneys are examined microscopically by an experimental pathologist. Immunohistochemical assessments are performed by expert microscopists utilizing immunoperoxidase-based immunohistochemistry and Image-Pro quantitative image analysis.

Parameters:
1. Mesangial proliferation (Ki-67 immunostaining), manual count, 25 random glomeruli.
2. Mesangial cell activation (alpha-smooth muscle actin, α-SMA immunostaining), image analysis, 25 random glomeruli.
3. Mesangial matrix expression (fibronectin), image analysis, 25 random glomeruli.
4. Inflammatory infiltration (ED-1, CD68 immunostaining), image analysis, 25 random glomeruli.

Sample Data:

Light microscopy (H&E): ATS induces a mesangioproliferative glomerulonephritis characterized by varying degrees of glomerular involvement from segmental to diffuse mesangial activation, proliferation and matrix synthesis.

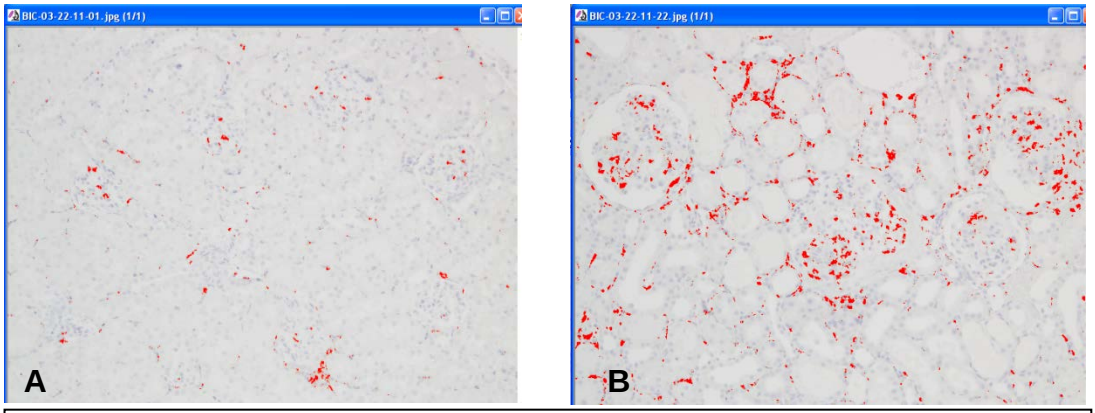


Mesangioproliferative glomerulonephritis in a rat kidney 5 days after injection of sheep anti-thymocyte (Thy-1) serum. (A): Glomerular mesangial hypercellularity and focal proliferative nodules are characteristic of the disease by H&E. (B): Mesangial cell activation is demonstrated by acquisition of α-SMA by immunofluorescence. Controls show negligible staining mainly in vessels (B). Glomeruli show strong α-SMA staining in nodules after ATS (C). Image analysis: Control: 0.74% of glomerular area, ATS: 31.7% of glomerular area

General Assessment (scoring criteria)

0 = Normal glomerular architecture;
1+ = Limited mesangial expansion or small nodules occupying combined area ≤ 1/3 glomerular area;
2+ = Mesangial expansion or nodules occupying a combined area of approximately 1/2 glomerular area;
3+ = Mesangial expansion or nodules occupying a combined area approximately 2/3 of glomerular area;
4+ = Segmental nodules and global mesangial hypercellularity.

Monocyte/macrophage (ED-1) infiltration (image analysis:



Macrophage infiltration during mesangioproliferative glomerulonephritis in a rat kidney 5 days after injection of sheep anti-thymocyte (Thy-1) serum. Macrophage infiltration is demonstrated by ED-1 immunoperoxidase staining followed by quantitative image analysis. Controls show basal staining (A). Glomeruli show markedly increased staining after ATS (B). Image analysis: Control: 1.74% section area, ATS: 6.6% of section area

Optional Endpoint Parameters

- PK/PD blood collections
- Sandwich ELISAs
- Clinical chemistry analysis
- Tissue harvesting
- Additional immunohistochemistry analysis
- Additional histopathologic analysis (image analysis)

Additional notes:

ATS (anti-Thy-1) is a very well characterized rat model of human mesangioproliferative glomerulonephritis and has been exceptionally useful in examining mechanisms of mesangial cell injury, mediators of proliferation, and extracellular matrix synthesis. Eloquent studies identified roles for PDGF, TGF-β and FGF in the pathogenesis of proliferation and matrix synthesis during disease progression. Moreover, the model has been used for the investigation of inflammatory response to glomerular injury. Mesangial cell apoptosis also occurs early and late in the disease and the model has been used to study programmed cell death in kidney disease. Other uses for the model are the examination mesangial cell response to injury and expression of α-smooth muscle actin, oxidative stress origin of glomerular cell and the progression of glomerulosclerosis or interstitial fibrosis, which may be elicited by multiple injections of the antisera a few days apart. Also the repeated injection of anti-Thy-1 and development of fibrosis may be analogous to persistent mesangial injury and progressive renal disease in

humans For more information about anti-Thy1 induced mesangial proliferative disease please visit our web site: www.probetex.com

Renal disease model descriptions:

http://www.probetex.com/uploads/3/5/3/9/3539181/immune-model-descriptions.pdf

References:

1. Glassock RJ, Adler SG, Ward HJ, Cohen AH. (1996). Primary glomerular diseases. In "The Kidney"(Brenner, B.M. Ed.) 5th ed. pp. 1182-1279. W. B. Saunders Company, Philadelphia.

2. Glassock RJ, Cohen AH, Adler SG, Ward HJ. (1996). Secondary glomerular diseases. In "The Kidney"(Brenner, B.M. Ed.) 5th ed. pp. 1182-1279. W. B. Saunders Company, Philadelphia.

3. Yamamoto T, and Wilson CB. Quantitative and qualitative studies of antibody-induced mesangial cell damage in the rat. Kidney Int 32:514-525, 1987.

4. Jefferson JA, Johnson RJ. Experimental mesangial proliferative glomerulonephritis (the anti-Thy-1.1 model). J Nephrol 12:297-307, 1999.

Please refer to the following publications by our clients using our services for this model:

McIntosh LM, Barnes JL, Barnes VL, McDonald JR. Selective CCR2-targeted macrophage depletion ameliorates experimental mesangioproliferative glomerulonephritis. *Clin Exp Immunol* 155:295-303, *2009. (**Contract Research, Pre-Clinical Testing, Histopathology)* Article: ***http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2249.2008.03819.x/epdf***

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