Rat Nephritogenic Monoclonal Antibodies to Glomerular Basement Membrane

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BACKGROUND

A novel rat nephritis model induced by monoclonal antibodies against the non-collagenous C-terminal domain (NC1) of the α4 chain of type IV collagen (α4(IV)NC1) was developed by Sado's group (1-2). Chondrex, Inc. provides three rat monoclonal antibodies against the α4(IV)NC1 of rat glomerular basement membrane (GBM), which can induce nephritis in Wistar-Kyoto rats by a single intraperitoneal (IP) or intravenous (IV) injection.

1) Monoclonal antibody, clone b35 (IgG2b), induces severe nephritis associated with hematuria and pulmonary hemorrhage.

2) Monoclonal antibody, clone a84 (IgG2a), induces severe nephritis associated with hematuria.

3) Monoclonal antibody, clone 114 (IgG1), induces mild nephritis at the same dose as b35 and a84.

It is believed that Goodpasture Syndrome, severe renal disease often associated with pulmonary hemorrhage, is mediated by autoantibodies against GBM. These autoantibodies target the NC1 domain of type IV collagen, which is a major constituent of basement membranes in kidney and lung tissues. Type IV collagen consists of six α-chains, designated α1(IV)-α6(IV). It has been reported that the primary Goodpasture antigen is located on α3(IV)NC1, even though antigenic epitopes expressed on α4(IV)NC1 and α5(IV)NC1 are recognized by autoantibodies from patients with Goodpasture Syndrome (3-5). On the other hand, it has been shown that α4(IV)NC1 is the strongest nephritogenic domain in rats, followed by α3(IV)NC1 and α5(IV)NC1, using both synthetic and recombinant peptides corresponding to human α3, α4, and α5(IV) NC1 (6-7). It is also reported that bovine and human α3(IV)NC1 can induce nephritis in mice (8-9). These differences in nephritogenic domains among animal species may depend upon the combinations of MHC haplotypes and species of NC1 used for immunization.

However, it is apparent that the role of non-MHC molecules is more critical in susceptibility to nephritis as various strains of rats and mice can produce autoantibodies to glomerular membranes, but do not develop nephritis. Also, these animals fail to induce nephritis when transferring autoantibodies to naïve animals.

NOTE: In normal glomerular development, the α1-α2 network is assembled first and then switched to α3, α4, and α5 networks (α6 is not located in the GBM) that then form the mature GBM in rodents and humans. On the other hand, Alport Syndrome is caused by a gene mutation at COL4A3, COL4A4, or COL4A5 where the GBM is composed of the embryonic α1-α2 network rather than the mature α3-α4-α5 network.

In order to study autoimmune-mediated nephritis, three classes of animal models have been developed. One is a passive rat nephritis model called Masugi Nephritis or nephrotoxic nephritis, which is induced by injecting rats with heterologous anti-GBM serum, such as rabbit anti-rat GBM serum (10). The next is a nephritis model called Steblay Nephritis, which is induced in animals such as sheep, guinea pigs, rabbits, and rats by actively immunizing them with heterologous or homologous GBM antigen emulsified with Complete Freund's Adjuvant (11-15). The third is a rat nephritis model induced by transferring purified antibodies from the urine of nephritic rats into naïve rats, indicating autologous antibodies can induce severe nephritis (16). As reagent preparation can be complicated, the use of these important animal models has been limited. From this viewpoint, monoclonal antibodies to autologous GBM are very important and useful tools in inducing nephritis in rats.

REAGENTS

Nephritogenic monoclonal antibodies: Affinity-purified rat monoclonal antibodies to α4 (IV) NC1 of rat GBM are provided at 1 mg/ml in 0.05M PBS pH 7.4.

Clone (Subtype)	Size	Catalog #
114 (IgG1)	1 ml	70201
	5 ml	70205
a84 (IgG2a)	1 ml	70211
	5 ml	70215
b35 (IgG2b)	1 ml	70221
	5 ml	70225

RECOMMENDED RAT STRAINS

Use young (7-8 weeks old), healthy WKY/NCrl (USA) or WKY/NCrlCrlj (Japan) rats and sub-strain SHR/NCrl rats (male or female) raised in specific pathogen free (SPF) conditions. For unknown reasons, these are the only strains highly susceptible to nephritis either by immunizing with GBM antigens or by injecting monoclonal antibodies against GBM (14-17). Other sub-strains from WKY rats, such as WKY/1zm or WKY/km are low responders to nephritis. In addition, the SHR/NCrl (spontaneous hypertension rats) strain is a high responder to monoclonal antibody (mAb)-induced nephritis, but the SHR/NHsd strain is resistant.

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Table 1 - Rat Strains Susceptible and Resistant to Nephritis

WKY Sub-Strains	Region	Susceptibility to mAb	Other strains	RT1 Type	Susceptibility to mAb
WKY/NCrlCrlj	Japan	High	Brown-Norway	N	Non
WKY/NCrl	USA	Medium-High	DA	A	Non
WKY/NlcoCrl	Europe	Low-Medium	PVG	C	Non
WKY/NHsd	USA	Non	LEW	L	Low
WKY/1zm	Japan	Low-Medium	WAG	U	Non
WKY/kw	Japan	Low-Medium	Wistar	Outbred	Low
SHR/NCrl	USA	High			
SHR/ NHsd	USA	Non			

NOTE 1: WKY/NlcoCrl (Europe), WKY/NHsd, WKY/1zm, and WKY/Kw rats are sub-strains of WKY rats but are assumed to be low responders or resistant to monoclonal antibody-induced nephritis for unknown reasons.

NOTE 2: Brown-Norway ($RT1^n$) rats (18) develop nephritis by immunizing with homologous and heterologous GBM, but they are resistant to mAb-induced nephritis. Other strains of rats, such as DA ($RT1^a$), PVG ($RT1^c$), LEW ($RT1^l$), and WAG ($RT1^u$) are low responders to glomerulonephritis by GBM immunization and monoclonal antibody injection (18).

NOTE 3: Three rat strains, Brown-Norway, Wistar, and LEW failed to develop nephritis after being injected with a cocktail of three monoclonal antibodies (114, a84, and b35). However, Wistar and LEW rats occasionally showed mild proteinuria associated with hematuria 2-3 weeks after injecting them with 1 mg of monoclonal antibody cocktail (0.333 mg each).

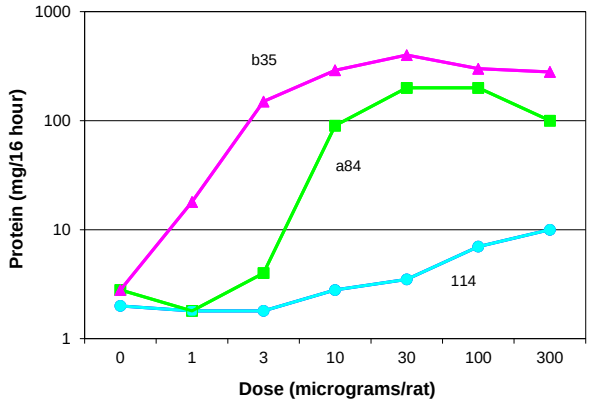
ADMINISTERING MONOCLONAL ANTIBODY

A single IV or IP injection of individual monoclonal antibodies in high responder rat strains can induce nephritis with or without pulmonary hemorrhage in a dose dependent manner (Figures 1 and 2) (1). Although nephritis can be induced in high responder strains with a very low dose of monoclonal antibodies (b35: 1-3 µg, a84: 3-10 µg, and 114: higher than 30 µg per rat), it is recommended to inject 100-300 µg of monoclonal antibody per rat in order to induce nephritis consistently. Total urinary protein amounts in a 16-hour collection period (6 pm-9 am) will increase within 2-3 days after the injection of monoclonal antibody (100 µg per rat), reach maximum levels at 8-10 days, and remain constant for 22 days (Figure. 3) (1).

Monoclonal antibody b35 will induce proteinuria, hematuria, and severe pulmonary hemorrhage at a dose of 300 µg per rat. Monoclonal antibody a84 will also induce proteinuria and hematuria at the same dose. On the other hand, 114 will bind to GBM, like b35 and a84, but will induce only mild nephritis without hematuria using the same dose ranges.

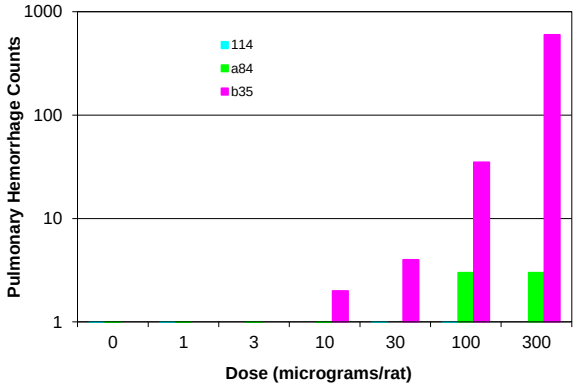
NOTE: Even at a lower dose (10 µg), a84 and b35 will induce moderate or severe proteinuria, which will remain for 22 days in WKY/NCrlCrlj rats. However, the urinary protein amounts in WKY/NcrlCrlj rats who received 10 µg of 114 peaked at day 8 and started to decrease by day 12.

Figure 1 - Dose response study of monoclonal antibodies in the induction of proteinuria.



NOTE: The protein amounts were determined on day 7 (data provided by Dr. Sado using WKY/NCrlCrlj rats).

Figure 2 - The development of pulmonary petechial hemorrhages depends upon the doses of monoclonal antibody.



NOTE: b35 induced severe pulmonary hemorrhages in the lungs and a84 induced similar pulmonary hemorrhages to a lesser extent. In contrast, 114 did not induce significant hemorrhages (data provided by Dr. Sado using WKY/NCrlCrlj rats).

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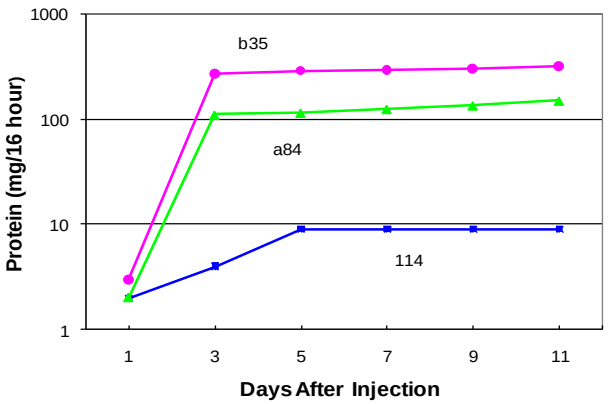
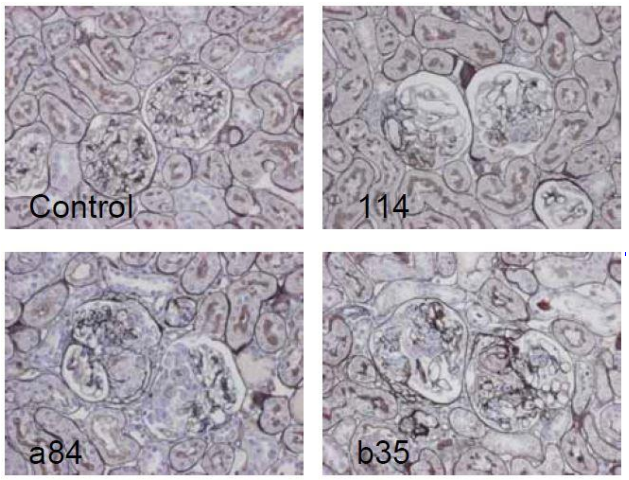
Figure 3 - Time course study of proteinuria after injecting 100 µg of individual monoclonal antibodies (data provided by Dr. Sado using WKY/NCrlCrlj rats).



HISTOLOGICAL CHANGES

Severe histological changes are observed in the kidneys after injecting nephritogenic monoclonal antibodies in high responder rats. For example, b35 (300 µg per rat) induced enlarged glomeruli with severe endocapillary hypercellularity and extra capillary changes such as capsular adhesion and crescent formation in 98% of glomeruli in rats on day 12 (Figure 4).Similarly, a84 (300 µg per rat) induced severe endocapillary hypercellularity and extra capillary changes such as capsular adhesion and crescent formation in 75% of glomeruli in rats. On the other hand, 114 (300 µg) only induced mild endocapillary and hypercellularity changes and small capsular adhesion in 7% of glomeruli in rats (1).

Figure 4 - Periodic acid-methenamine-silver (PAM) staining showing histological changes in the kidney on day 12 after individual monoclonal antibody injections (300 µg) in rats (data provided by Dr. Sado using WKY/NCrlCrlj rats).

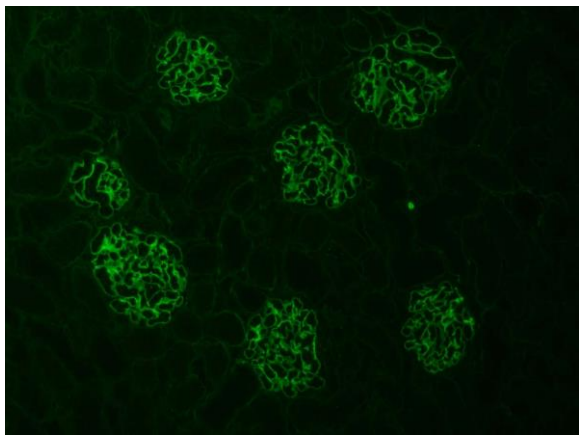


DEPOSITION OF MONOCLONAL ANTIBODIES ALONG GBM AND TUBULAR BASEMENT MEMBRANES (TBM)

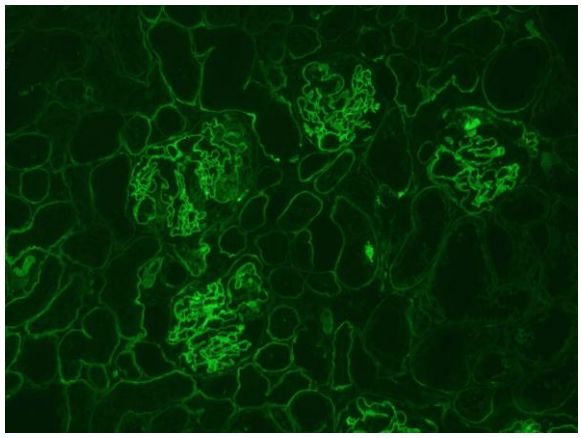
The deposition of monoclonal antibodies on GBM and TBM in the high responder rat strains show a dose dependent manner. At low doses of monoclonal antibodies (114: 100 µg, a84: 10 µg, and b35: 10 µg), linear deposition was observed along the GBM (Figure 5A). However, at higher doses for a84 and b35 (more than 100 µg), additional antibody deposition on the TBM is observed. Figure 5B shows antibody deposition on GBM and TBM from b35 (100 µg) injected rats which induced severe pulmonary hemorrhage (1).

Figure 5 - Direct immunofluorescence showing linear deposition of 114 on the GBM (A) and of b35 on glomerular and tubular basement membranes (TBM) (B) of rats who received 100 µg antibody injections (data provided by Dr. Sado using WKY/ NCrlCrlj rats).

(A)



(B)



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URINE COLLECTION

As rat urine volumes vary in individual rats, Chondrex Inc recommends evaluating total protein amount in a pooled urine collection, not urine protein concentration. Collect urine every other day from 6 pm to 9 am with metabolic cages. Measure the urine volume and centrifuge to remove insoluble materials. Keep the supernatant in a refrigerator for short-term storage and –20°C for long-term storage.

ASSAY FOR URINARY PROTEIN

Urinary protein concentrations in samples can be determined by either the turbidity protein assay method using 3% sulfosalicylic acid dihydrate (19), the Bradford protein assay method using Coomassie brilliant blue, the Rat Albumin Detection ELISA Kit (Cat # 3020), or the BCA (Bicinchoninic Acid) protein assay. Regardless of which assay method is used, BSA cannot be used as a standard because the dose response curve of BSA in assays significantly differs from the curve generated by serum proteins. The optical density (OD) value of immunoglobulins in the Bradford protein assay is only 70% of the OD value generated by BSA. Use a standard protein solution prepared from normal rat serum. Chondrex, Inc. recommends the turbidity assay method in micro-titer plates for assaying a large number of urine samples due to the stability of turbidity, the wide ranges of protein concentration, and the wide range of the linear dose response curve (protein concentration from 0.05 to 4 mg/ml). Please refer to Chondrex, Inc.'s Rat Urinary Protein Assay Kit (Cat # 9040) and Rat Albumin Detection Kit (Cat # 3020) for more detailed information.

NOTE: Data should be interpreted as the total amount of urinary protein collected in 16 hours instead of protein concentration, as the protein concentration is occasionally very high when the urinary volume is low. Therefore, Chondrex, Inc. strongly recommends not using protein assay strips for assaying rat urinary protein levels. For example, the total volume of urine collected from rats in a 16 hour period varies significantly from 0.7 ml to 20 ml depending upon individual strains under normal conditions, and protein levels in low urinary volume samples will be judged as 2+ (30 mg/dl), 3+ (100 mg/d) or 4+ (more than 2000 mg/dl) even in normal rats. However, the total amount of protein in urine collected in 16 hours is less than 5 mg compared to the 100 mg range seen in nephritogenic rats.

ASSAY FOR HEMATURIA

Hematuria test paper such as Hema-Combistix, Bayer-Sankyo Japan or other similar methods can be used. In addition, Chondrex, Inc. provides a Hemoglobin Assay Kit (Cat # 6024).

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