Rat Nephritogenic Monoclonal Antibodies to Glomerular Basement Membrane

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

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

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

3) Monoclonal antibody, 114 (IgG1), induces mild nephritis at the same dose of 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 NC1 domain of type IV collagen, which is a major constituent of basement membrane 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 the NC1 domain of the α3 chain of type IV collagen [α3(IV)NC1] [3-4], even though antigenic epitopes expressed on the α4(IV)NC1 and α5(IV)NC1 chains are recognized by autoantibodies from patients with Goodpasture syndrome [5]. On the other hand, it has been shown that α4 is the strongest nephritogenic fragment in rats followed by α3 and α5(IV)NC1, using both synthetic and recombinant peptides corresponding to α3, α4 and α5(IV) of human NC1 [6-7]. It is also reported that α3 of bovine and human type IV collagen NC1 fragment can induce nephritis in mice [8-9]. These differences in nephritogenic fragment among species of animals may depend upon the combinations of MHC haplotypes of animals and species of NC1 fragments used for immunization.

However, it is apparent that the role of non-MHC molecules is more critical in susceptibility to nephritis, since various strains of rats and mice are capable of producing autoantibody to glomerular membrane, but do not develop nephritis. Also, these animals are resistant to antibody-transfer nephritis.

NOTE: In normal glomerular development, the α1-α2 network is assembled first and then switched to α3, α4 and α5 network (α6 is not located in the GBM) that forms the mature GBM in rodents and humans. On the other hand, Alport syndrome is caused by a gene mutation at COL4A3, COL4A4 or COL4A5, and 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 nephritis model called Masugi Nephritis or nephrotoxic nephritis, which is induced in rats by injection of heterologous anti-GBM sera, such as rabbit anti-rat GBM sera [10]. The next is a model called Steblay nephritis, which is induced in animals such as sheep [11], guinea pigs [12], rabbits [13-14] and rats [15-16] by active immunization with heterologous or homologous GBM antigen emulsified with Complete Freund's Adjuvant. The third is a model induced by passive transfer of purified antibodies from the urine of nephritic rats, indicating autologous antibodies are capable of inducing severe nephritis [17]. However, glomerulonephritis cannot be transferred into normal rats by injecting sera from nephritic rats. This is because autoantibodies are rapidly consumed by binding to autologous GBM and secreted into the urine with other serum proteins, resulting in low serum concentration levels. Regardless of the types of model used for research, it is difficult to obtain highly purified antigenic NC1 fragment of type IV collagen from GBM, thus usage 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.

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Reagents

Nephritogenic monoclonal antibodies: Each vial contains a 1 mg/mL PBS solution of affinity-purified rat monoclonal antibody to α4 (IV) NC1 of rat GBM.

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

Recommended Rat Strains

Use young (7-8 weeks old), healthy WKY/NCrl (in USA), WKY/NCrlCrlj (in Japan) or WKY/NlcoCrl (in Europe) rats and sub-strains SHR/NCrl rats (male or female) raised in SPF conditions. For unknown reasons, these are the only strains known to be highly susceptible to nephritis both by immunization with GBM antigen and by injecting monoclonal antibodies against GBM [15-18] and other sub-strains from WKY rats, such as WKY/1zm, WKY/km are low responder to nephritis. In addition, SHR/NCrl (Spontaneous hypertension rats) strain is high responder to mAb-induced nephritis but SHR/NHsd strain is resistant.

Table 1 - Rat strains susceptible and resistant to nephritis

WKY Sub-Strains	Note (Region)	Susceptibility to mAb	Other Strains	Note (RT1 Type)	Susceptibility to mAb
WKY/NCrlCrlj	Japan	High	Brown-Norway	$RT1^n$	Non
WKY/NCrl	USA	High	DA	$RT1^a$	Non
WKY/NlcoCrl	Europe	High	PVG	$RT1^c$	Non
WKY/NHsd	USA	Non	LEW	$RT1^l$	Low
WKY/1zm	Japan	Low-Medium	WAG	$RT1^u$	Non
WKY/kw	Japan	Medium	Wistar	Outbred	Low
SHR/NCrl	USA	High			
SHR/NHsd	USA	Non			

NOTE 1: WKY/NHsd, WKY/1zm, WKY/Kw rats are sub-strains of WKY rats, but are assumed to be low responder or resistant to monoclonal-induced nephritis for unknown reasons.

NOTE 2: Brown-Norway ($RT1^n$) rats [19], which is reported to be a responder to homologous and heterologous GBM and develops nephritis, was resistant to monoclonal antibody-induced nephritis. Other strains of rats, such as DA ($RT1^a$), PVG ($RT1^c$), LEW ($RT1^l$) and WAG ($RT1^u$) are unlikely to be susceptible to glomerulonephritis by active immunization [19] and monoclonal antibody injection.

NOTE 3: Three strains of rats, Brown-Norway, Wistar and LEW rats were tested for the susceptibility to a cocktail of three monoclonal antibodies (114, a84 and b35), but no apparent signs of nephritis was observed in these strains. However, mild proteinuria associated with hematuria was observed occasionally in Wistar and LEW rats 2-3 weeks after injection of 1 mg of monoclonal antibody cocktail (0.333 mg each).

Administration of Monoclonal Antibody

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

Monoclonal antibody b35 will induce proteinuria and hematuria, and also severe pulmonary hemorrhage when 300 μg of the antibody per rat is used. Monoclonal antibody a84 will also induce proteinuria and hematuria. On the other hand, 114 will bind to GBM as well as b35 and a84, but induce only mild nephritis without hematuria at 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 levels in WKY/NcrlCrlj rats receiving 10 μg of 114 reached the peak at day 8 and started to decrease on day 12.

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Figure 1 - Dose response study of monoclonal antibodies in the induction of proteinuria. **NOTE:** The protein levels were determined on day 7 (data provided by Dr. Sado using WKY/NCrlCrlj rats).

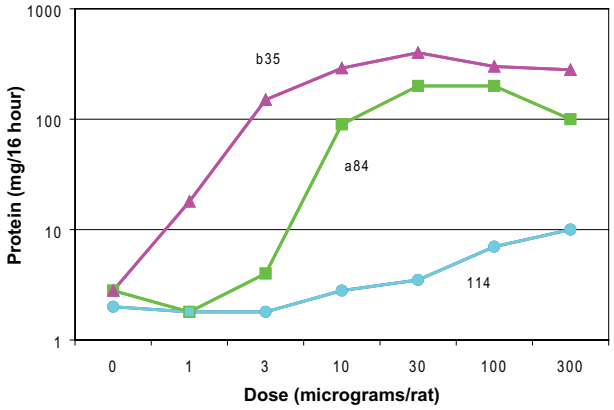


Figure 2 - The development of pulmonary petechial hemorrhages depends upon the doses of monoclonal antibody. **NOTE:** b35 induced severe pulmonary hemorrhages at the lung and a84 induced similar pulmonary hemorrhages but to a lesser extent. In contrast, 114 did not induce significant hemorrhages (data provided by Dr. Sado using WKY/NCrlCrlj rats).

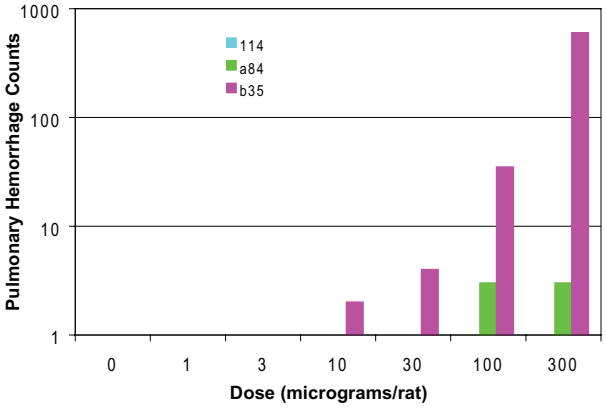
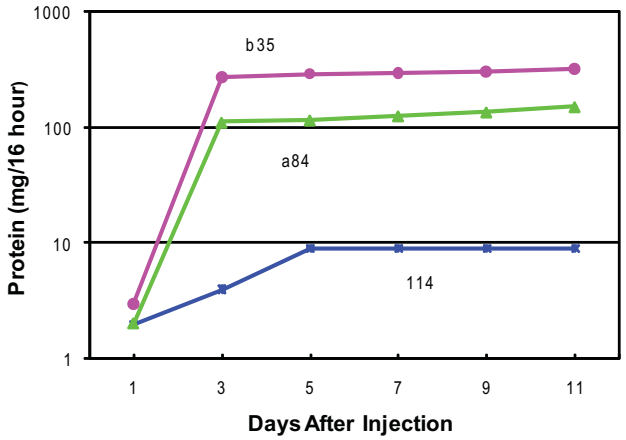


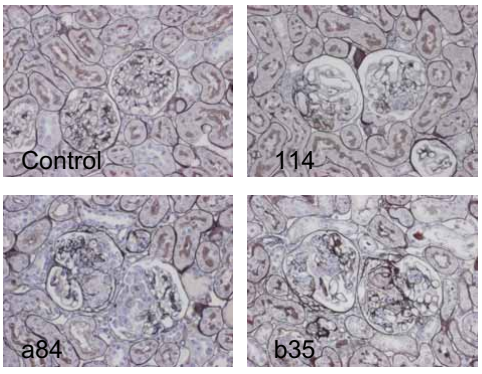
Figure 3 - Time course study of proteinuria after injection of 100 μg of individual monoclonal antibodies (data provided by Dr. Sado using WKY/NCrlCrlj rats).



Histological Changes

Severe histological changes are observed in the kidneys after injection of nephritogenic monoclonal antibodies (Fig. 4) in the high responder strains. For example, enlarged glomeruli with severe endocapillary hypercellularity and extracapillary changes such as capsular adhesion and crescent formation is observed in 98% of glomeruli in rats 12 days after injection of b35 (300 μg per rat). Similarly, severe endocapillary hypercellularity and extracapillary changes such as capsular adhesion and crescent formation in 75% of glomeruli in rats receiving a84 (300 μg). On the other hand, mild endocapillary hypercellularity and small capsular adhesion in 7% of glomeruli in rats receiving 114 (300 μg) was observed [1].

Figure 4 - Periodic acid-methenamine-silver (PAM) staining showing histological changes in the kidney from rats 12 days after injection of 300 μg of individual monoclonal antibodies (data provided by Dr. Sado using WKY/NCrlCrlj rats).



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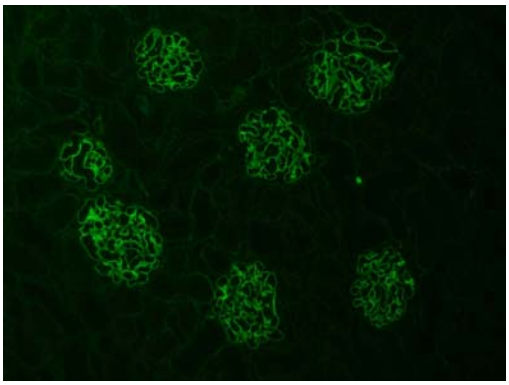
Deposition of Monoclonal Antibodies Along GBM and Tubular Basement Membranes (TBM)

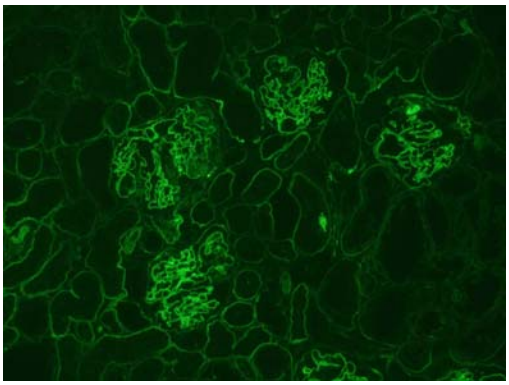
The deposition of monoclonal antibodies on GBM and TBM in the high responder strains is dose dependent. At low doses of monoclonal antibodies (114: 100 μg, a84: 10 μg and b35: 10 μg), linear deposition is observed along the GBM (Fig. 5A). However, at higher doses of a84 and b35 (more than 100 μg), additional deposition of monoclonal antibodies on TBM is observed, moreover this is most apparent in the kidney from rats receiving b35 (Fig. 5B), which causes severe pulmonary hemorrhage [1].

Figure 5 - Direct immunofluorescence showing linear deposition of 114 on glomerular basement membrane and of b35 on glomerular and tubular basement membrane (data provided by Dr. Sado using WKY/NCrlCrlj rats). (A) 114 - 100 μg per rat (B) b35 - 100 μg per rat.

(A)



(B)



Urine Collection

Collect urine from 5 pm to 9 am every other day 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

Protein concentrations in urine samples will be determined by both turbidity protein assay method using 3% sulfosalicylic acid dihydrate [20] and Bradford protein assay method using coomassie brilliant blue. Regardless of which assay method is used, BSA cannot be used as a standard, because the dose response curve of BSA in turbidity assay significantly differs from the curve of serum proteins, and OD value of globulins in Bradford protein assay is only 70% of the OD value of BSA. Use a standard protein solution prepared from normal rat serum. We recommend turbidity assay method in micro-titer plates for assaying a large number of urine samples with wide ranges of protein concentration, because of the wide range of linear dose response curve, and the stability of turbidity. One regression curve ($Y=aX^2+bX$) can be used to calculate protein concentration from 0.05 to 4 mg/mL. Please refer to the Rat Urinary Protein Assay Kit (Catalog #9040) for more detailed information.

NOTE: Data should be explained as total amount of proteins in 16 hour urine samples instead of protein concentration, since the protein concentration is occasionally very high when the urinary volume is low. Therefore, we strongly recommend not using protein assay strips for assaying rat urinary protein levels. For example, the 16 hour urinary volume 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/dL) or 4+ (more than 2000 mg/dL) even in normal rats. However, the total amount of proteins in a 16 hour urine is less than 5 mg compared to 100 mg range in nephritogenic rats.

Assay for Hematuria

Use a hematuria test paper such as Hema-Combistix, Bayer-Sankyo Japan or other convenient methods.

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