Inducing Experimental Autoimmune Glomerulonephritis in WKY Rats

Catalog # 1102

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INTRODUCTION

Goodpasture's syndrome is an autoimmune disease which can induce rapidly progressive glomerulonephritis (RPDN) and lung hemorrhage (1, 2) and is mediated by autoantibodies to the basement membrane of glomeruli and alveoli. Type IV collagen, the major component of the glomerular basement membrane (GBM), is composed of five α(IV) chains, α1 (IV) to α5 (IV) (3, 4). The non-collagenous (NC1) domain of the α3 chain of type IV collagen (α3 (IV) NC1) was considered a main Goodpasture antigen in humans (5-10).

Experimental autoimmune glomerulonephritis (EAG) as a clinical model of Goodpasture's syndrome can be induced in Wistar Kyoto (WKY) rats by immunizing them with heterologous and homologous GBM (11), the NC1 fraction of type IV collagen (4,12,13), purified bovine α3 (IV) NC1 (12,13), recombinant human α3 (IV) NC1 and α4 (IV) NC1 (14-16), and synthetic peptides of human α3 (IV) NC1 (17-20). These results suggest that Goodpasture antigens in rats are located on α3 (IV) NC1 and α4 (IV) NC1 (15). EAG can also be induced in naïve WKY rats by transferring either autoantibodies (21) or T-cells specific to peptides of α3 (IV) NC1 (22) from nephritic WKY rats, indicating that anti-GBM antibodies and antigen-specific T-cells would be independently involved in the pathogenesis of EAG.

Chondrex, Inc. provides the bovine NC1 fraction of type IV collagen (Cat # 1102, 1 mg), which is isolated from collagenase solubilized bovine GBM, and can be used as one of the Goodpasture's antigens to induce EAG in WKY rats. A Rat Urinary Protein Assay Kit (Cat # 9040) is also available to evaluate the total protein amounts in 16-hour urine in EAG rats. Alternatively, a BPB Protein Assay Kit (Cat # 6026) can work to evaluate proteinuria and a Creatinine Assay Kit (Cat # 6041) can be used to normalize the amounts of proteinuria or albuminuria.

PROTOCOL FOR INDUCING EAG

1. **Animals**: 7-8 week or older WKY rats. Sensitivity to EAG may vary depending on the sub-strain of WKY rat, animal vendor, or facility.

2. **Antigen**: Dissolve one vial of NC1 fraction of type IV collagen (Cat # 1102, 1 mg) in 1 ml of 0.05M phosphate buffered saline, pH 7.4 (PBS).

3. **Emulsion**: Make an emulsion using 1 mg/ml of NC1 fraction with an equal volume of Complete Freund's Adjuvant (Cat # 7008 - *M. Tuberculosis*, 1 mg/ml).

   NOTE: Check the stability of the emulsion by adding one drop of emulsion into a beaker of water. If the emulsion remains as a solid clump on the water's surface and does not dissipate, the emulsion is considered stable. If the emulsion spreads onto the water surface, add a few drops of adjuvant, mix again, and retest.

4. **Immunization**: Inject 0.2 ml (NC1 fraction - 0.1 mg) of the emulsion, subcutaneously at the base of the rat tail.

   NOTE: A booster injection on day 7 may be effective for inducing EAG in low responder rats. Incomplete Freund's Adjuvant (IFA) (Cat # 7008) should be used to prepare the emulsion for a booster injection instead of CFA. Subcutaneously inject 0.2 ml (NC1 fraction - 0.1 mg) of the emulsion at the base of the rat tail. Each investigator must determine the optimal conditions for each experiment, such as immunization schedule and doses for effectively inducing nephritis in individual strains of rats, as the susceptibility to nephritis varies even among the same strain of rats from different animal vendors.

5. **Evaluating EAG**: After immunizing the rats, Chondrex, Inc. recommends observing the rats until day 35. Each week perform 16-hour proteinuria evaluations to determine the severity of EAG. Chondrex, Inc.'s Rat Urinary Protein Assay Kit (Cat # 9040) is recommended for the proteinuria assay. Proteinuria is usually observed on day 14 - 21 in susceptible WKY rats and reaches a maximum level on day 21 - 28.

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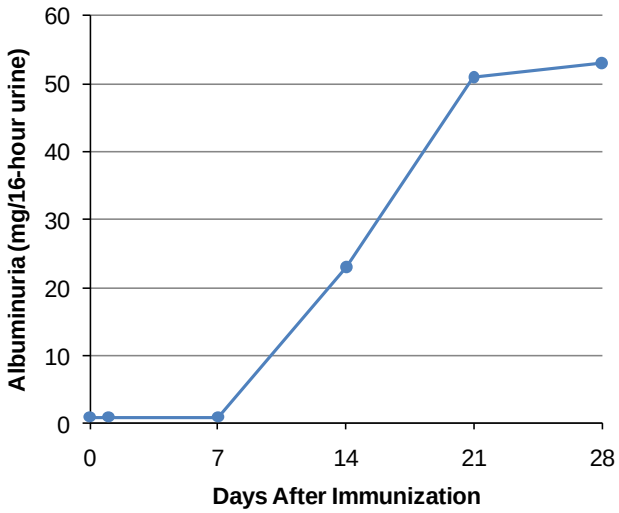
Experimental Autoimmune Glomerulonephritis Induced by Immunization with Bovine NC1 Fraction in WKY Rats



WKY rats (females, 7 weeks old from Harlan Laboratories, USA) were immunized with 100 µg of bovine NC1 fraction (Cat # 1102) emulsified with CFA (Cat # 7008, *M. Tuberculosis*, 1 mg/ml) by subcutaneous injection at the base of the tail on day 0. The rats developed proteinuria on day 10, which lasted until day 28.

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