

Induction of Experimental Autoimmune Glomerulonephritis in WKY Rats

Catalog # 1102 (NC1 fraction of bovine type IV collagen)

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INTRODUCTION

Goodpasture's syndrome, known as an autoimmune disease inducing rapidly progressive glomerulonephritis (RPDN) and lung hemorrhage (2, 19), 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, α 1 (IV) to α 5 (IV) (7,16). The non-collagenous (NC1) domain of the α 3 chain of type IV collagen (α 3 (IV) NC1) was considered a main Goodpasture antigen in humans (5, 6, 9, 11, 12, 20). Experimental autoimmune glomerulonephritis (EAG) in Wistar Kyoto (WKY) rats used as a clinical model of Goodpasture's syndrome can be induced by immunizing heterologous and homologous GBM (14), NC1 fraction of type IV collagen (13, 16, 17), purified bovine α 3 (IV) NC1 (1, 10), recombinant human α 3 (IV) NC1 and α 4 (IV) NC1 (1, 10, 15), and synthetic peptides of human α 3 (IV) NC1 (3, 4, 8, 21). EAG can also be induced in naïve WKY rats by transferring either autoantibodies (18) or T-cells specific to peptides of α 3 (IV) NC1 (22) from nephritic WKY rats, indicating that anti-GBM antibodies and antigen-specific T-cells would be involved independently in the pathogenesis of EAG. Chondrex provides the NC1 fraction of type IV collagen (catalog # 1102, 1 mg), which are isolated from collagenase-solubilized bovine GBM, and can be used as one of the Goodpasture's antigen to induce EAG in WKY rats.

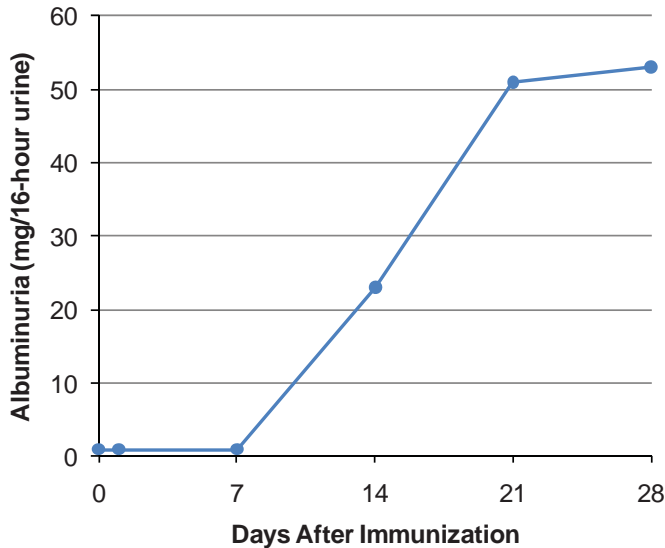
PROTOCOL FOR INDUCING EAG

1. **Animals:** 7-8 weeks 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 in 1 ml of PBS.
3. **Emulsion:** Make an emulsion using 1 mg/ml of NC1 fraction with an equal volume of complete Freund's adjuvant (catalog # 7008 - M. Tuberculosis, 1 mg/ml).

Note: Check the stability of emulsion by adding one drop of emulsion into a beaker of water. If the emulsion remains as a solid clump which does not dissipate, the emulsion is considered stable. If the emulsion spreads onto the water surface, add a few drop 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.
5. **Evaluation of EAG:** Measure a 16-hour proteinuria for determining the severity of EAG once a week after immunization. Chondrex's Rat Urinary Protein Assay Kit (catalog # 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. We recommend observing the rats until day 35.

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 (catalog # 1102) emulsified with CFA (catalog # 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 and lasted until day 28.

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