Inducing Mouse Immune Complex Glomerulonephritis by Cationic Bovine Serum Albumin

Catalog # 9058

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INTRODUCTION

Membranous nephropathy (MN) is a disease caused by granular subepithelial deposition of immunocomplexes along the glomerular basement membrane (GBM) in kidneys. The pathogenesis of MN is still unknown in humans; however, it is believed to be associated with other diseases such as systemic lupus erythematosus, hepatitis, and cancers. As a human MN disease model, immune complex glomerulonephritis (ICGN) can be induced in rats by daily intravenous (IV) administrations of 2 mg of cationic bovine serum albumin (cBSA) for 4 weeks (1). The positively charged cBSA has been shown to be effective at inducing ICGN due to its ability to penetrate and bind to the fixed negatively charged sites found in the GBM of the kidneys (2-4). Additionally, ICGN animal models by cBSA injection have been successfully established in dogs, cats, rabbits, and mice (5-8).

Chondrex, Inc. provides cBSA (Cat # 9058) for inducing ICGN in inbred Balb/c and outbred ICR mice. The Mouse Urinary Albumin Detection ELISA Kit (Cat # 3012) is the most effective assay to evaluate ICGN severity, as a typical symptom of ICGN is albuminuria. Alternatively, the BPB Protein Assay Kit (Cat # 6026) works to evaluate proteinuria and the Creatinine Assay Kit (Cat # 6041) can be used to normalize the amounts of proteinuria or albuminuria.

PROTOCOL FOR INDUCING ICGN

1. **Animals**: 7-8 week or older Balb/c or ICR mice.

2. **Antigen**: 2 mg/ml cBSA, 10 ml 0.05 phosphate buffered saline, pH 7.4 (PBS).

3. **Emulsion**: Make an emulsion using 2 mg/ml of cBSA 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 0.1 ml of the emulsion (cBSA: 0.1 mg), subcutaneously at the base of the mouse tail.

5. **Inducing Nephritis**: Two weeks after the immunization, inject 0.2 ml (400 µg) of cBSA (2 mg/ml) solution intravenously. Repeat the IV injections every other day, for a total of 5 IV injections.

   NOTE: IV injection may cause an anaphylactic reaction, which is lethal in mice. To avoid this problem, use slow IV injections (1 ml/minute) or larger volumes with lower cBSA doses or concentrations. For example, dilute cBSA to the appropriate concentration with PBS. Then use 50 - 100 µg (0.5 -1 ml of 0.1 mg/ml cBSA) for the first injection and 400 µg (1 ml of 0.4 mg/ml cBSA) for the second to fifth injections.

6. **Evaluating ICGN**: Evaluate the severity of ICGN by determining the amount of albumin excreted during a 16-hour urine collection period (albuminuria). Chondrex, Inc.'s Mouse Urinary Albumin Detection ELISA Kit (Cat # 3012) is recommended.

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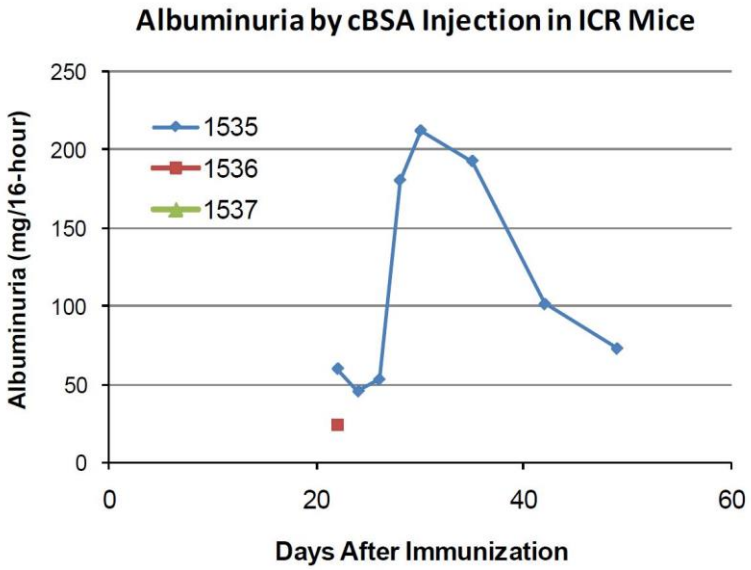
SAMPLE DATA – ICGN IN ICR MICE

1. 7-8-week-old ICR mice (males, Harlan, USA) were used.
2. Mice received 100 µg of cBSA emulsified with CFA (Cat # 7008, *M. Tuberculosis*, 1 mg/ml) by subcutaneous injection at the base of the tail on day 0.
3. 400 µg (0.2 ml of 2 mg/ml) of cBSA was injected intravenously into mice on day 14, 16, 18, 20, and 22.
4. Albuminuria was evaluated using a 16-hour urine collection period in metabolic cages.



In mice, albumin amounts greater than 1 mg of albumin in a 16-hour urine collection period is considered albuminuria. Mouse # 1537 died after the first cBSA IV injection on day 14. Mouse # 1536 died on day 24 after the first urine collection. Mouse # 1535 developed severe albuminuria, peaking on day 32.

SAMPLE DATA – ICGN IN BALB/C MICE

1. 7-8-week-old Balb/c mice (males, Harlan, USA) were used.
2. Mice received 100 µg of cBSA emulsified with CFA (Cat # 7008, *M. Tuberculosis*, 1 mg/ml) by subcutaneous injection at the base of the tail on day 0.
3. 50 µg (0.1 ml of 0.5 mg/ml) of cBSA was injected intravenously into mice on day 14.
4. 400 µg (0.2 ml of 2 mg/ml) of cBSA was injected intravenously into mice on day 16, 18, 20, and 22.
5. Albuminuria was evaluated using a 16-hour urine collection period in metabolic cages.

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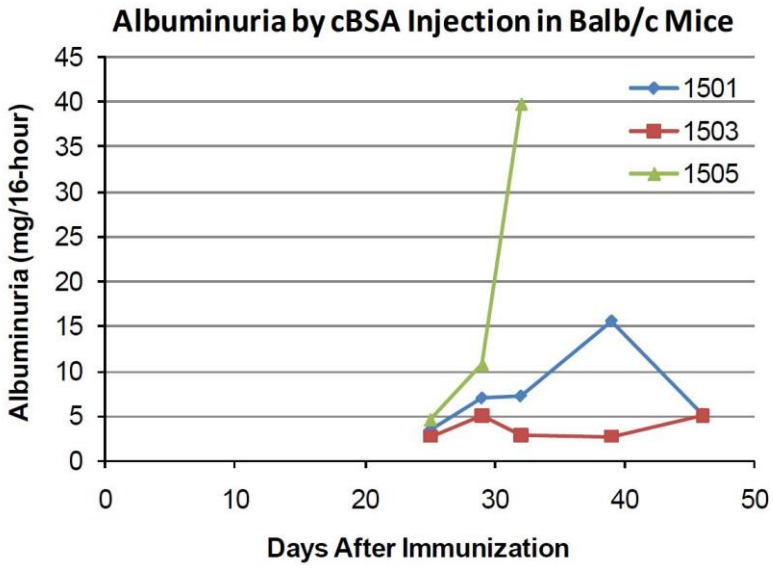

By day 24, all mice had developed albuminuria. Mouse # 1505 showed a high level of albuminuria and died on day 30. Albuminuria lasted for 3 weeks after the last cBSA IV injection in the other two mice. Mouse # 1501 showed peak albuminuria levels on day 39. Mouse # 1503 consistently leaked lower levels of urine albumin compared to the other mice.

REFERENCES

1. T. Yamamoto, I. Kihara, T. Morita, T. Oite, Bovine serum albumin (BSA) nephritis in rats. I. The Experimental Model. *Acta Pathol Jpn* **28**, 859-66 (1978).

2. T. Yamamoto, S. Miyazaki, K. Kawasaki, E. Yaoita, I. Kihara, Rat Bovine Serum Albumin (BSA) Nephritis. VI. The Influence of Chemically Altered Antigen. *Clin Exp Immunol* **65**, 51-6 (1986).

3. W. Border, E. Kamil, H. Ward, A. Cohen, Antigenic changes as a determinant of immune complex localization in the rat glomerulus. *Lab Invest* **45**, 442-9 (1981).

4. T. Oite, S. Batsford, M. Mihatsch, H. Takamiya, A. Vogt, Quantitative Studies of in Situ Immune Complex Glomerulonephritis in the Rat Induced by Planted, Cationized Antigen. *J Exp Med* **155**, 460-74 (1982).

5. N. Wright, N. Mohammed, P. Eckersall, A. Nash, Experimental immune complex glomerulonephritis in dogs receiving cationized bovine serum albumin. *Res Vet Sci* **38**, 322-8 (1985).

6. Nash, N. Mohammed, N. Wright, Experimental Immune Complex Glomerulonephritis and the Nephrotic Syndrome in Cats Immunised With Cationised Bovine Serum Albumin. *Res Vet Sci* **49**, 370-2 (1990).

7. P. Bass, Y. Wang, N. al, B. Evans, H. Thomas, D. Davies, *et al.*, The effect of cyclosporin A on cationized bovine serum albumin-induced nephropathy in NZW rabbits. *J Pathol* **167**, 41-7 (1992).

8. J. Chen, A. Chen, L. Chang, W. Chang, H. Lee, *et al.*, Mouse Model of Membranous Nephropathy Induced by Cationic Bovine Serum Albumin: Antigen Dose-Response Relations and Strain Differences. *Nephrol Dial Transplant* **19**, 2721-8 (2004).

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