

Induction of 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. A human MN disease model induces immune complex glomerulonephritis (ICGN) in rats by daily intravenous (IV) administrations of 2 mg of cationic bovine serum albumin (cBSA) for 4 weeks (1).

cBSA has been shown to be effective at inducing ICGN due to the negatively fixed charged sites found in the GBM (2). These negatively charged sites may increase the penetration and binding of positively charged substances in the kidney such as cBSA (3, 4). Additionally, ICGN animal models by cBSA injection have been successfully established in dogs (5), cats (6), rabbits (7), and mice (8).

Chondrex, Inc. provides cBSA (catalog # 9058) for inducing ICGN in inbred Balb/c and outbred ICR mice. A Mouse Albumin Detection Kit (catalog # 3012) is also available to evaluate ICGN severity, as a typical symptom of ICGN is 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 PBS.
3. **Emulsion:** Make an emulsion using 2 mg/ml of cBSA with an equal volume of complete Freund's adjuvant (catalog # 7008 - *M. Tuberculosis*, 1 mg/ml).

Note: Check the stability of the emulsion by adding one drop of emulsion to a beaker of water. If the emulsion remains as a solid clump which does not dissipate, the emulsion is considered stable. If the emulsion dissipates on the water's surface, add a few drops of adjuvant to the remaining emulsion, mix, and retest.

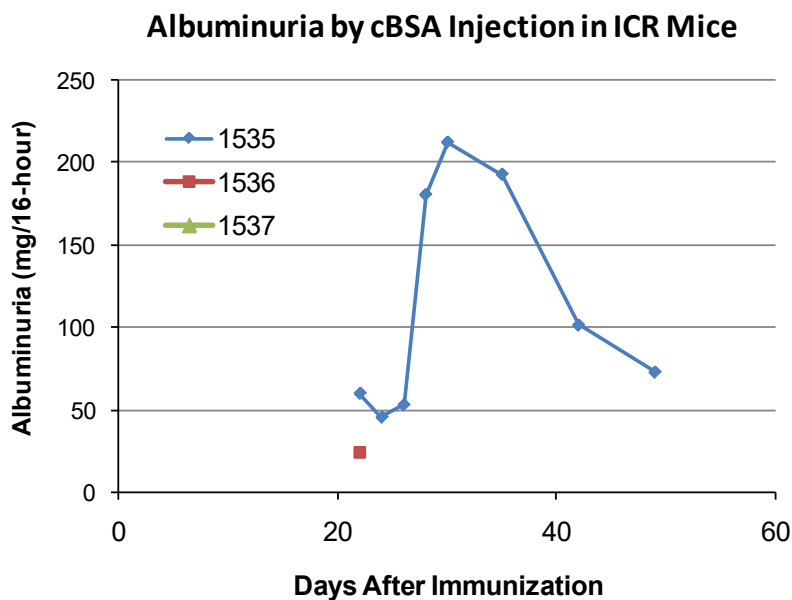
4. **Immunization:** Inject 0.1 ml of the emulsion (cBSA: 0.1 mg), subcutaneously at the base of the mouse tail.
5. **Induction of nephritis:** Two weeks after the immunization, inject 0.2 ml (400 µg) of cBSA (2 mg/ml) solution intravenously. Repeat the IV injections every other day, for a total of 5 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 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. **Evaluation of nephritis:** Evaluate the severity of nephritis by determining the amount of albumin excreted during a 16-hour urine collection period (albuminuria). Chondrex, Inc.'s Mouse Albumin Detection Kit (catalog # 3012) is recommended.

### SAMPLE DATA - ICGN IN ICR MICE

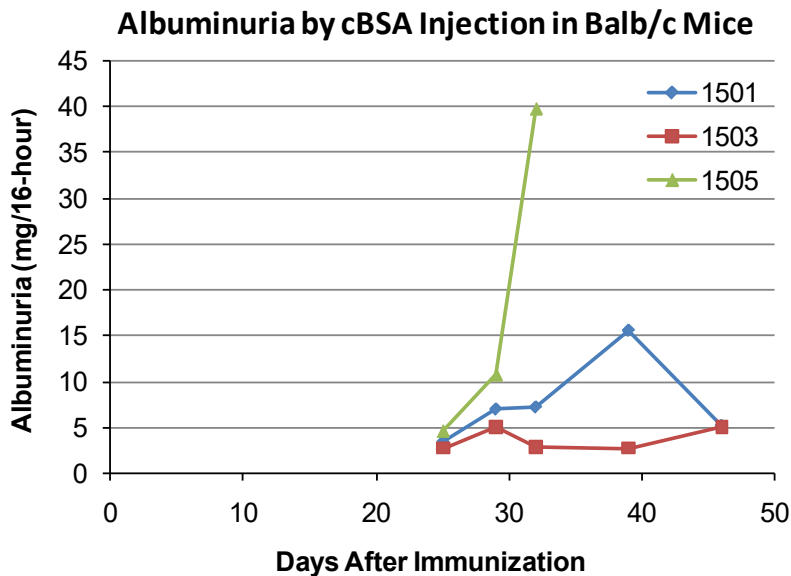
1. 7-8 week old ICR mice (males, Harlan, USA) were used.
2. Mice received 100 µg of cBSA emulsified with CFA (catalog # 7008, *M. Tuberculosis*, 1 mg/ml) by subcutaneous injection at the base of the tail on day 0.
3. 400 µg (0.2 ml of 2 mg/ml) of cBSA was injected intravenously into mice on day 14, 16,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



In mice, any albumin amount greater than 1 mg of albumin in a 16-hour urine collection period is considered albuminuria. Mouse # 1537 was killed by the first cBSA IV injection on day 14. Mouse # 1536 was dead 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 weeks old Balb/c mice (males, Harlan, USA) were used.
2. Mice received 100  $\mu$ g of cBSA emulsified with CFA (catalog # 7008, *M. Tuberculosis*, 1 mg/ml) by subcutaneous injection at the base of the tail on day 0.
3. 50  $\mu$ g (0.1 ml of 0.5 mg/ml) of cBSA was injected intravenously into mice on day 14.
4. 400  $\mu$ g (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 from mice in metabolic cages.



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

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