

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 patients. However, it is believed that MN is associated with other diseases such as, systemic lupus erythematosus, hepatitis, and cancers. Immune complex glomerulonephritis (ICGN) in rats used as a human MN disease model was established by daily intravenous (IV) administration of 2 mg of cationic bovine serum albumin (cBSA) for 4 weeks (1). More recently, cBSA has been shown to be more 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 such as cBSA in the kidney (3, 4). Additionally, ICGN animal models by cBSA injection have been successfully established in dogs (5), cats (6), rabbits (7), and mice (8).

Chondrex provides cBSA (catalog # 9058, 20 mg, 2 mg/ml in PBS pH 7.4, 10 ml) 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 weeks or older Balb/c or ICR mice. Sensitivity to ICGN may vary depending on the strain of mice, animal vendor, or facility.
2. **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 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.

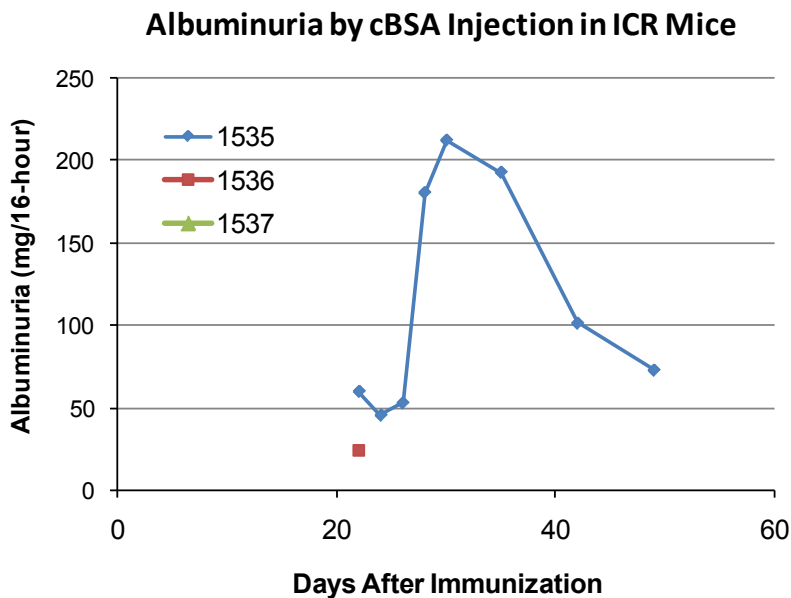
3. **Immunization:** Inject 0.1 ml of the emulsion (cBSA: 0.1 mg), subcutaneously at the base of the mouse tail.
4. **Induction of nephritis:** Two weeks after the immunization, inject 0.2 ml (400 μ g) of cBSA (2 mg/ml) solution intravenously. Repeat the IV injections every other day, for a total of 5 times.

Note: The IV injection of cBSA may cause an anaphylaxis reaction, which is lethal to mice. In this case, use a lower dose (50 – 100 μ g of cBSA) for the first injection, and then increase the dose to 400 μ g of cBSA for the second to fifth injections.

5. **Evaluation of nephritis:** Evaluate the severity of nephritis by determining the amount of albumin excreted during a 16-hour urine collection (albuminuria). Chondrex's Mouse Albumin Detection Kit (catalog # 3012) is recommended.

SAMPLE DATA - ICGN IN ICR MICE

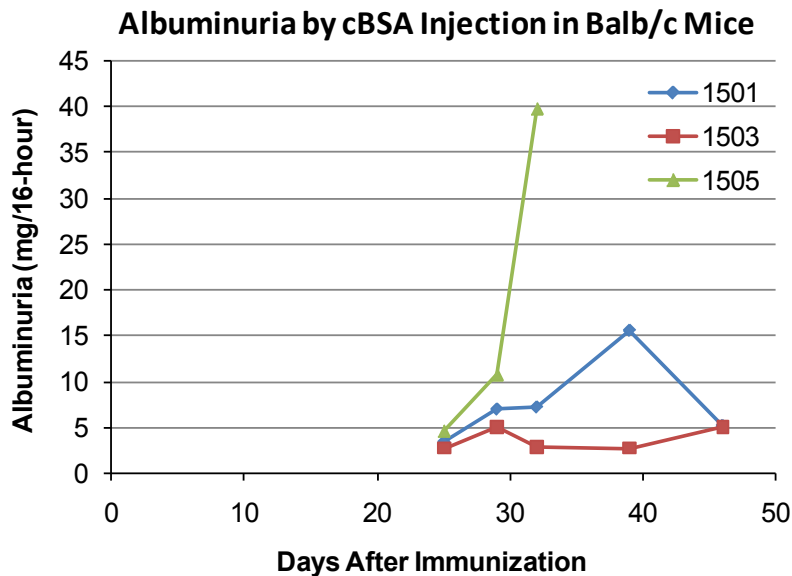
1. 7-8 weeks old ICR mice (males, Harlan, USA) were used.
2. Mice received 100 μ g of cBSA emulsified with CFA (catalog # 7008, M. Tuberculosis, 1 mg/ml) by subcutaneous injection at the base of the tail on day 0.
3. 400 μ g (0.2 ml of 2 mg/ml) of cBSA was injected intravenously in mice on day 14, 16,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



Generally, greater than 1 mg of albumin in a 16-hour urine collection is considered as albuminuria in mice. One mouse (# 1537) was killed by the first cBSA IV injection on day 14. One mouse (# 1536) was dead on day 24 after the first urine collection. The remaining mouse (# 1535) developed severe albuminuria with a peak 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 μ 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 μ g (0.1 ml of 0.5 mg/ml) of cBSA was injected intravenously in mice on day 14.
4. 400 μ g (0.2 ml of 2 mg/ml) of cBSA was injected intravenously on day 16, 18, 20, and 22.
5. Albuminuria was evaluated using a 16-hour urine collection from mice in metabolic cages.



On day 24, all mice had already developed albuminuria. Mouse # 1505, possessed high 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 on day 39. Mouse # 1503 consistently leaked lower level albumin in urine than other mice.

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