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(54) HB_cAb HUMAN PLASMA

(71) We, MERCK & CO INC., a corporation duly organized and existing under the laws of the State of New Jersey, United States of America, of Rahway, New Jersey, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed to be particularly described in and by the following statement:

This invention is concerned with the production of HB_cAb human plasma, i.e. human plasma containing antibody (HB_cAb) to hepatitis B core antigen (HB_cAg) in high yield and purity.

Hepatitis B is one of the types of viral hepatitis which results in a systemic infection with the principal pathologic changes occurring in the liver. This disease affects mainly adults and is maintained chiefly by transfer of infection from long term carriers of the virus. Usual methods of spread are by blood transfusion, contaminated needles and syringes, through skin breached by cuts or scratches, by unsterilized dental instruments as well as by saliva, veneral contact or exposure to aerosolized infected blood.

The incubation period of type B hepatitis is relatively long: from 6 weeks to 6 months may elapse between infection and the onset of clinical symptoms. The illness usually begins with fatigue and anorexia, sometimes accompanied by myalgia and abdominal discomfort. Later jaundice, dark urine, light stools and tender hepatomegaly may appear. In some cases, the onset may be rapid, with appearance of jaundice early in association with fever, chills, and leukocytosis. In other cases jaundice may never be recognized and the patient may be aware of a "flu-like" illness. It is estimated that the majority of hepatitis infections result in a mild, anicteric illness.

Serum obtained from patients with hepatitis B infections usually contains three distinct morphologic forms. The largest of these morphologic forms, a 42-nm to 45-nm double shelled spherical particle, often referred to as the Dane particle (HBV), is believed to be the virus of hepatitis B. The outer surface or envelope of the Dane particle (HB_sAg) surrounds a 27-nm inner core which does not react with antibody against HB_sAg and which contains a distinct antigen, the core antigen (HB_cAg). Antibody to HB_cAg appears after acute hepatitis B infection, and also can be demonstrated consistenty in chronic carriers of HB_sAg. Highly sensitive techniques are now available for detection of the HB_cAg system. A deterrent to the more widespread use of such techniques, however, is the absence of a simple yet practical and effective method for obtaining HB_cAg. The methods proposed heretofore generally involve the use of selected plasma which contains exceptionally high amounts of Dane particles.

In accordance with the present invention, there is provided a method of preparing HB_cAb human plasma having at least 2000 IAHA units/ml (IAHA means "immune adherence hemagglutination assay") comprising administering HB_cAg at predetermined intervals to a human individual positive for HB_cAb, and thereafter plasmapheresing the individual when the HB_cAb titer is elevated.

The following Example is illustrative. Percentages are by weight.

EXAMPLE

A. *Preparation of Dane particles (HBV)*

The rotor of a centrifuge, Electronucleonics K is filled with 8,400 ml of phosphate buffer. After running the rotor up to 10,000 rpm to degas the system, the following step gradient is

pumped into the bottom of the stationary rotor:

1. 2,400 ml of 10% NaBr, ρ = 1.08
2. 1,000 ml of 20% NaBr, ρ = 1.17
3. 1,500 ml of 30% NaBr, ρ = 1.28
4. 3,500 ml of 40% NaBr, ρ = 1.41

Plasma containing HB_sAg, 1,750 ml, is pumped into the top of the stationary rotor displacing 1,750 ml of 40% NaBr from the bottom of the rotor. The rotor is accelerated to 30,000 rpm and run at this speed for 4 hours. The rotor is then stopped and 1,750 ml of 40% NaBr are pumped into the bottom of the rotor forcing the plasma out of the top. An additional 1,750 ml of fresh plasma containing HB_sAg are pumped into the top of the rotor displacing an equal volume of 40% NaBr out the bottom of the rotor. The rotor is then run at 30,000 rpm for 18 hours. After stopping the rotor 1,000 ml of Dane particle rich material in the 1.26 - 1.30 density region is collected.

The Dane particles (HBV) are separated from the NaBr zonal fraction in the following procedure. The zonal fraction (1000 ml) is diluted to 3000 ml using phosphate buffered saline. This material is then placed into twelve type 19 rotor plastic bottles (ea. 250 ml/bottle). The material is then centrifuged using a type 19 rotor (Beckman). The rotor is spun at 17,000 rpm (45,000 × g) for 24 hours in order to pellet the Dane particles. The rotor is then stopped and the supernate from each bottle is decanted. The pellet material from all 12 bottles is recovered in a total volume of 5-7 ml of Tris-saline buffer and stored at −70°C. This material is the Dane particle concentrate.

B. *Purification of Dane particles*

1 ml of concentrated Dane particles from part A is layered over 4 ml of 20% sucrose - 1% bovine serum albumin (BSA) in Tris buffer (pH 7.6) in a SW 65 rotor with 1/2 × 2" cellulose nitrate tubes. The particles are centrifuged at 35,000 rpms for 4 hours. Post centrifugation, the supernate fluid is decanted and the pellet is gently resuspended in 0.5 ml of Tris buffer with 1% BSA using a cotton tipped swab (pre-moistened with buffer). The cotton swab is then rinsed with 0.5 ml of buffer. The final volume of Dane particle is 1 ml. The Dane particles are stored at −70°C.

C. *Preparation of HB_cAg (core antigen)*

The material from part B, 1 ml, is added to 1 ml of a 1% (v/v) solution of 2-mercaptoethanol in deionized water, and 1 ml of a 1% (v/v) solution of polyoxyethylene (20) sorbitan monooleate in deionized water. The resulting mixture is agitated gently and replaced in a 37°C water bath. After 1 hour the mixture is diluted with TMN-1% BSA (a solution containing 0.08 M Tris, 0.008 M $MgCl_2$ and 0.14 M NaCl, and 1% BSA) using a previously calculated quantity of diluent until it contains 32 IAHA units per ml. The solution is then dispensed into plastic 2 ml screw-cap serum tubes (0.5 ml/tube) and stored in a liquid nitrogen freezer.

D. *Adsorption on alum*

10 ml of the HB_cA_g (Type Ad) containing 16-32 IA units/ml are mixed with 0.85 ml of 10% alum solution $KA1(SO_4)_2.12H_2O$. While stirring 0.1 N NaOH is added slowly to adjust the pH to 6.8. Mixing is continued for 1 hour at room temperature. The solution is then centrifuged at 1500 × g for 10 minutes. The supernate is decanted and the pellet is resuspended with saline solution to the original volume (10 mls). The solution is then mixed for 5-10 minutes prior to use as an antigen.

This procedure is repeated with 10 ml of HB_cAg (Type Ay).

E. *Production of HB_cAb sera*

I. The alum antigens prepared as in D are used to make high-titer HB_cAb sera. The guinea pigs are divided into two groups which are used to produce the HB_cAb antiserum. The first group is administered intramuscular injections of 3 doses of 0.5 ml at monthly intervals of the product from HB_cAg (Type Ad), and the second group is treated similaly with the product from HB_cAg (Type Ay). High-titred hepatitis B antibody sera are produced in each group.

II. The product from C is treated under aseptic conditions with 1:4000 formalin at 37°C for 72 hours. Excess formalin is then neutralized with sodium bisulfate. The core antigen is then adsorbed an alum by following the procedure of Step D.

Individuals positive for HB_cAb and having an HB_cAb antibody titer (as measured by the immune adherence hemagglutination assay, IAHA) of 32 IAHA units/ml or greater are administered 1 ml (40 μg) doses of vaccine intramuscularly. Additional injections are given

1 month and 3 months following the first injection. One week after the third injection the individuals are plasmapheresed and HB_cAb titers are run on the individual plasma using the immune adherence hemagglutination assay. The majority of the individuals experience an increase in their HB_cAb titer compared to their initial titer. Those plasmas having an antibody titer of 2000 or higher are processed to yield gamma globulin having high HB_cAb titer.

The specification of our copending application No. 15228/78 (Serial No. 1596591) describes and claims the production of HB_cAg by treating Dane particles with a nonionic surfactant having from 15 to 35 oxyethylene units in the presence of a thiol reducing agent to remove the surface antigen from the Dane particle.

The specification of our copending application No. 7944611 (Serial No. 1596593) describes and claims a composition comprising hepatitis B core antigen in a medium consisting essentially of TMN-1% BSA as hereinbefore defined, saline or carbonate buffer.

WHAT WE CLAIM IS:

1. A method of preparing HB_cAb human plasma having at least 2000 IAHA (as hereinbefore defined) units/ml comprising administering HB_cAg at predetermined intervals to a human individual positive for HB_cAb, and thereafter plasmapheresing the individual when the HB_cAb titer is elevated.

2. A method according to claim 1 in which a gamma globulin fraction is recovered from the plasma.

3. A method according to claim 1 substantially as hereinbefore described in the Example.

4. HB_cAb human plasma having at least 2000 IAHA (as hereinbefore defined) units/ml prepared by administering HB_cAg at predetermined intervals to a human individual positive for HB_cAb, and thereafter plasmapheresing the individual when the HB_cAb titer is elevated.

5. Gamma globulin recovered from HB_cAb human plasma according to claim 1.

6. HB_cAb human plasma obtained by a method substantially as hereinbefore described in the Example.

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