PATENT SPECIFICATION (11) 1 596 593

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(21) Application No. 44611/79 (22) Filed 18 Apr. 1978

(62) Divided Out of No. 1596591

(31) Convention Application No. 789033 (32) Filed 20 Apr. 1977 in

(33) United States of America (US)

(44) Complete Specification Published 26 Aug. 1981

(51) INT.CL.[3] A61K 39/29

(52) Index at Acceptance
A5B 120 132 134 136 137 AA

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(54) HEPATITIS B CORE ANTIGEN

(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 relates to hepatitis B core antigen (HB_cAg).

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 milk, 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 of 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 consistently 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.

The preparation of HB_cAg is isopycnic banding of biological fluid from human HB_sAg positive donors, optionally but preferably pelleting the Dane particles, and then removing the surface antigen by contacting the Dane particles which a nonionic surfactant having from 15 to 35 oxyethylene units in the presence of a reducing agent such as mercaptoethanol, is described and claimed in the specification of our copending application No. 15228/78 (Serial No. 1596591). Attention is also directed to the specification of our copending application No. 7923093 (Serial No. 1596592).

The present invention provides a composition comprising hepatitis B core antigen in a medium consisting essentially of TMN-1% BSA as hereinafter defined, saline or carbonate buffer, e.g. SPGA. Also in accordance with the present invention, such a composition is inactivated to produce a vaccine effective to produce hepatitis B core antibody, which is another embodiment of the present invention. Inactivation may be effected by an aqueous solution of formaldehyde and the core antigen may be

adsorbed on a physiologically acceptable substrate, e.g. alum.

In the following Examples which illustrate the invention, all percentages are by weight, the term "IAHA" means "immune adherence agglutination assay" and "SPGA" is a stabilizer composed of sucrose, phosphate, glutamine and albumin disclosed by Bovarnick et al, J. Bact. *59* (1950) pp 509-521.

Example 1

Dane particles are prepared and purified by the method of Example 1 of the specification of our copending application No. 15228/78 (Serial No. 1596591). Then the surface antigen is removed as described in that Example by treatment with 2-mercaptoethanol and polyoxyethylene (20) sorbitan monooleate. The resulting mixture is agitated gently and placed in a 37°C water bath. After 1 hour the mixture is diluted with TMN-1% BSA (which means a solution containing 0.08 M Tris, 0.008 M $MgCl_2$ and 0.14 M Na Cl, 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.

Example 2

HB_cAg prepared as in Example 1 of specification No. 15228/78 (Serial No. 1596591) is adsorbed on alum as follows. 10 ml of HB_cAg (Type Ad) containing 16-32 IA units/ml is mixed with 0.85 ml of 10% of alum solution $KAl(SO_4)_2.12H_2O$. 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 but using 10 ml of HB_cAg (type Ay) instead of HB_cAg (type Ad).

Example 3

The final product of Example 1 is treated under aseptic conditions with 1:4000 formalin at 37°C for 72 hours. Excess of formalin is then neutralized with sodium bisulfite. The core antigen is then adsorbed on alum by following the procedure of Example 2.

Example 4

The material from part B of Example 1 of specification No. 15228/78, 1 ml, is added to 1 ml of a (v/v) solution of 2-mercaptoethanol in dionized 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 placed in a 37°C water bath. After 1 hour the mixture is diluted with SPGA 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.

WHAT WE CLAIM IS:-

1. A composition comprising hepatitis B core antigen in a medium consisting essentially of TMN-1% BSA as hereinbefore defined, saline or carbonate buffer.
2. A vaccine effective to produce hepatitis B core antibody comprising an inactivated composition according to Claim 1.
3. A vaccine according to Claim 2 in which the inactivation is effected by an aqueous solution of formaldehyde.
4. A vaccine according to Claim 2 in which the medium is SPGA as hereinbefore defined.
5. A vaccine according to Claim 2 in which the core antigen is adsorbed on a physiologically acceptable substrate.
6. A vaccine according to Claim 4 in which the substrate is alum.
7. A method of preparing a vaccine effective to induce formation of antibody to hepatitis B core antigen that comprises inactivating a composition according to Claim 1.
8. A method according to Claim 7 in which the treated hepatitis B core antigen is adsorbed on alum.
9. A composition as claimed in Claim 1 substantially as hereinbefore described in Example 1.
10. A composition as claimed in Claim 2 substantially as hereinbefore described in Example 2 or 4.
11. A method as claimed in Claim 7 substantially as hereinbefore described in Example 3.

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Printed for Her Majesty's Stationery Office,
by Croydon Printing Company Limited, Croydon, Surrey, 1981.
Published by The Patent Office, 25 Southampton Buildings,
London, WC2A 1AY, from which copies may be obtained.

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7. A method of preparing a vaccine effective to induce formation of antibody to hepatitis B core antigen that comprises inactivating a composition according to Claim 1.
8. A method according to Claim 7 in which the treated hepatitis B core antigen is adsorbed on alum.
9. A composition as claimed in Claim 1 substantially as hereinbefore described in Example 1.
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