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(54) **HUMAN MONOCLONAL ANTIBODY AGAINST HEPATITIS B VIRUS SURFACE ANTIGEN (HBVSAG)**

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A61K 39/395 (2006.01)

(52) **U.S. Cl.** **424/149.1**; 424/130.1; 424/141.1; 424/133.1; 424/142.1; 424/161.1; 435/70.21; 435/326; 435/339

(58) **Field of Classification Search** 424/149.1, 424/130.1, 133.1, 142.1, 161.1; 435/70.21, 435/326, 339
See application file for complete search history.

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(74) *Attorney, Agent, or Firm*—Browdy and Neimark, PLLC

(57) **ABSTRACT**

Disclosed is a hybridoma cell line which produces human antibodies capable of binding to the hepatitis B virus surface antigen (HBVsAg), as well as antibodies produced by the cell line. Also disclosed are various uses of said antibodies in the prevention and treatment of HBV infection. Peripheral blood lymphocytes obtained from human donors having a high titer of anti HBVsAg antibodies are activated in vitro with pokeweed mitogen and then fused with heteromyeloma cells to generate hybridomas secreting human antibodies having a high affinity and specificity to HBVsAg.

12 Claims, 7 Drawing Sheets

FIG. 1A

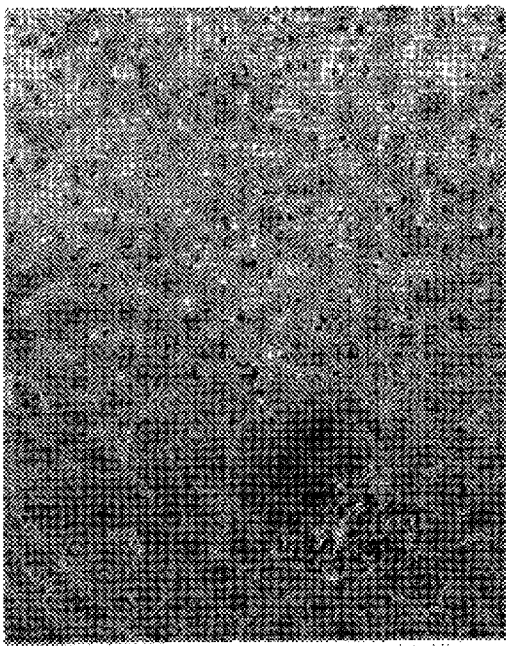
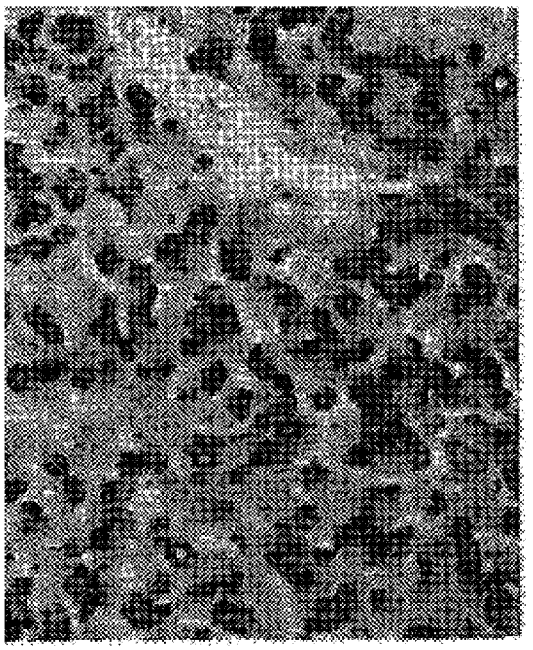


FIG. 1B



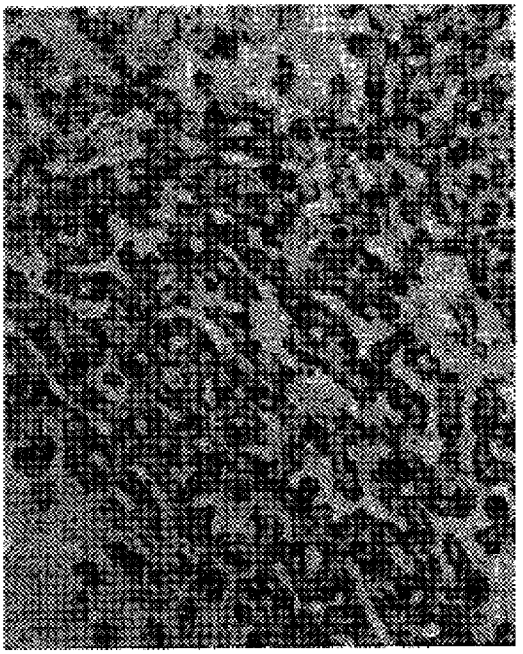


FIG. 1C

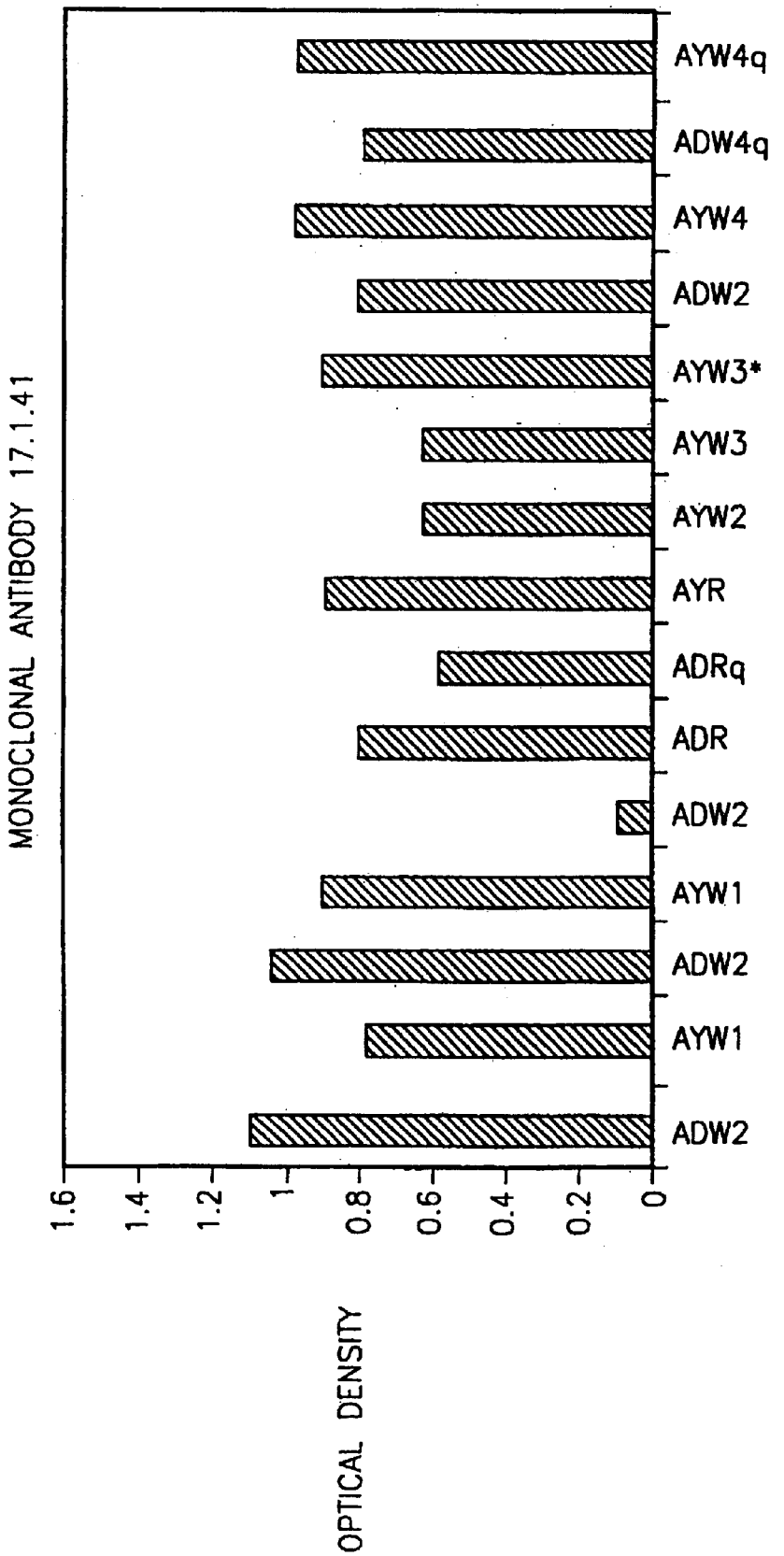

MONOCLONAL ANTIBODY 17.1.41
AYW4q
ADW4q
AYW4
ADW2
AYW3*
AYW3
AYW2
AYR
ADRq
ADR
ADW2
AYW1
ADW2
AYW1
ADW2
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
OPTICAL DENSITY

FIG.2

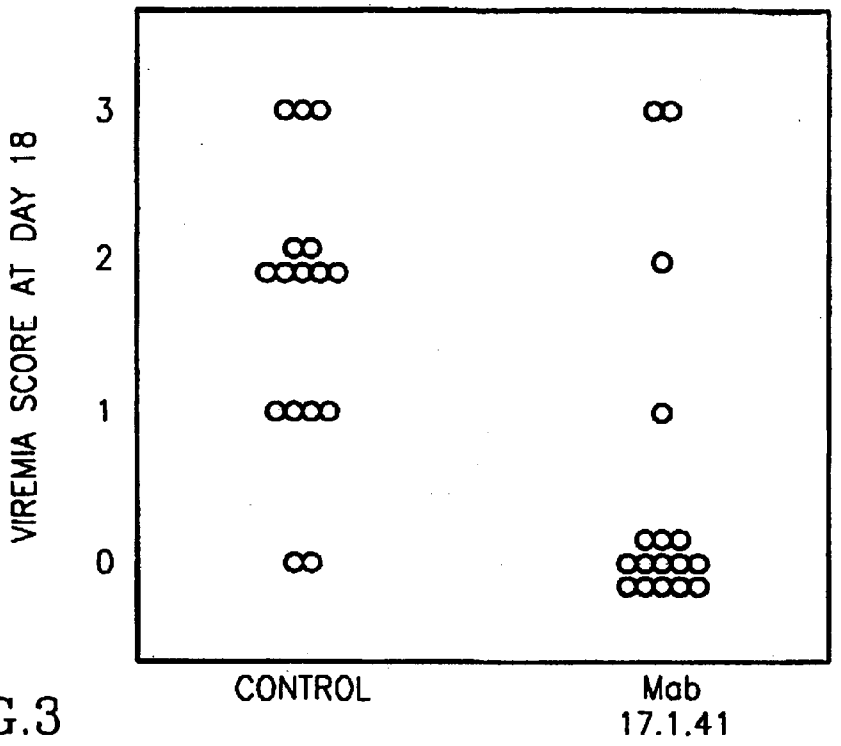

VIREMIA SCORE AT DAY 18
3
2
1
0
CONTROL
Mab
17.1.41

FIG.3

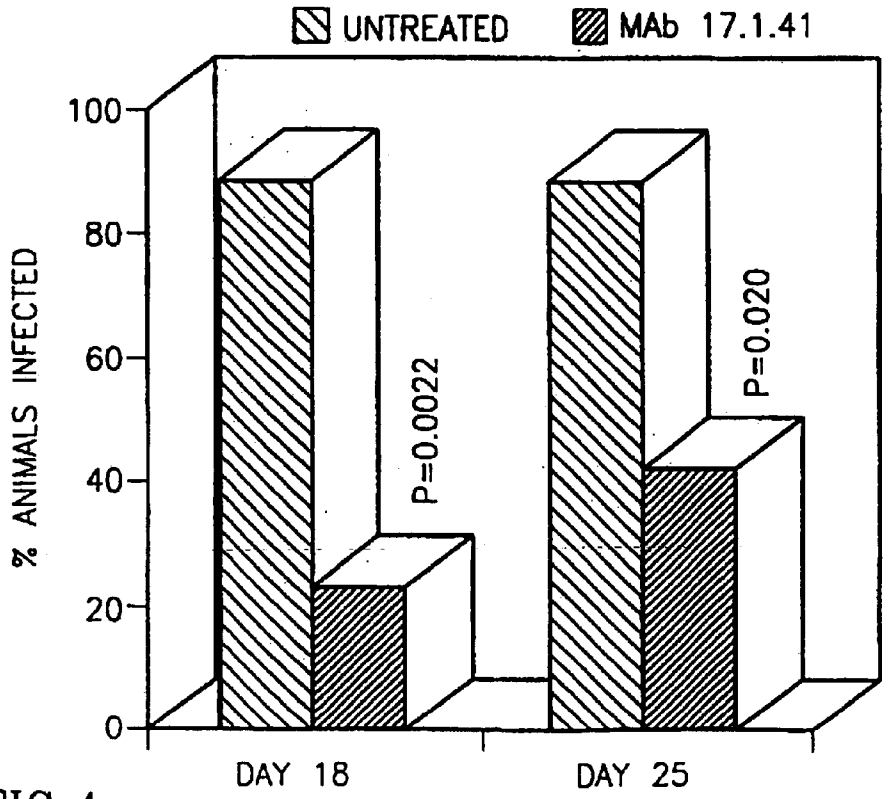

UNTREATED
MAb 17.1.41
% ANIMALS INFECTED
100
80
60
40
20
0
P=0.0022
P=0.020
DAY 18
DAY 25

FIG.4

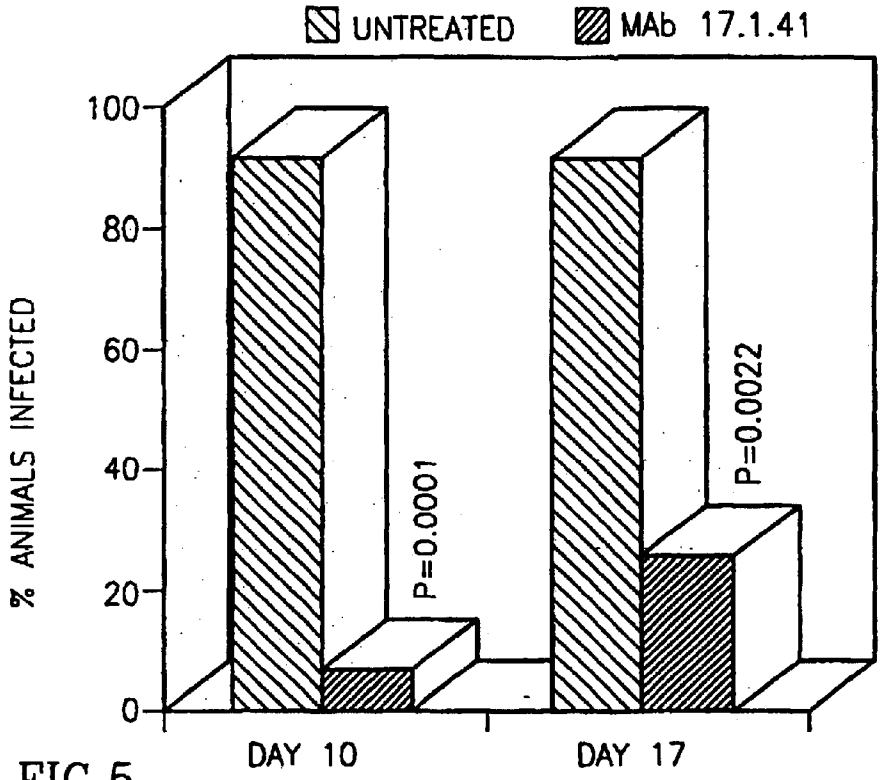

UNTREATED
MAb 17.1.41
% ANIMALS INFECTED
100
80
60
40
20
0
P=0.0001
P=0.0022
DAY 10
DAY 17

FIG.5

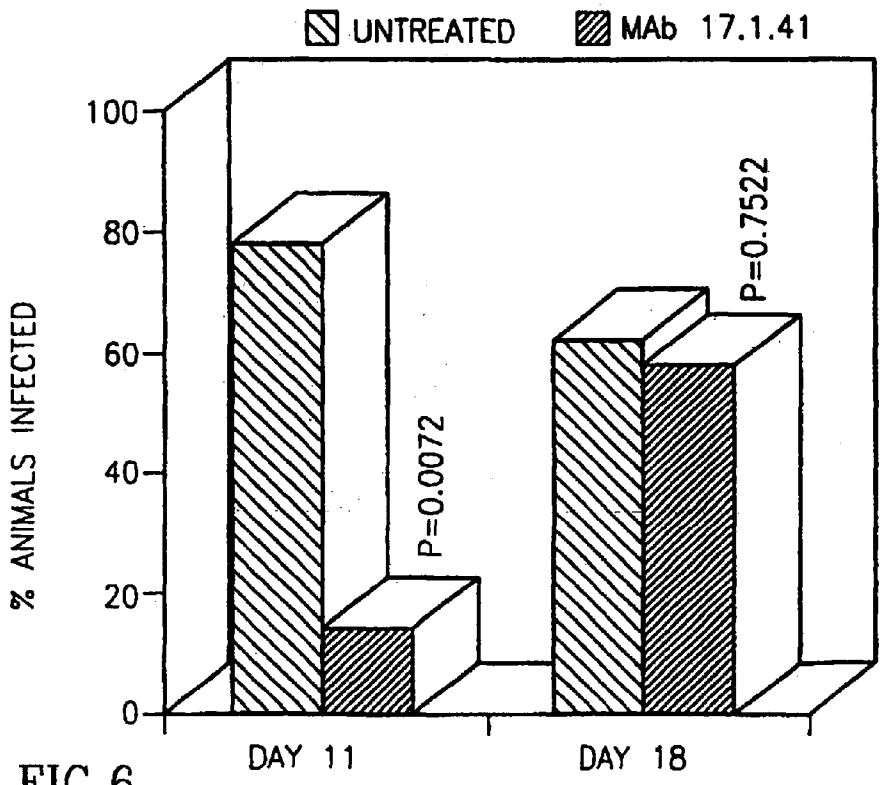

UNTREATED
MAb 17.1.41
% ANIMALS INFECTED
100
80
60
40
20
0
P=0.0072
P=0.7522
DAY 11
DAY 18

FIG.6

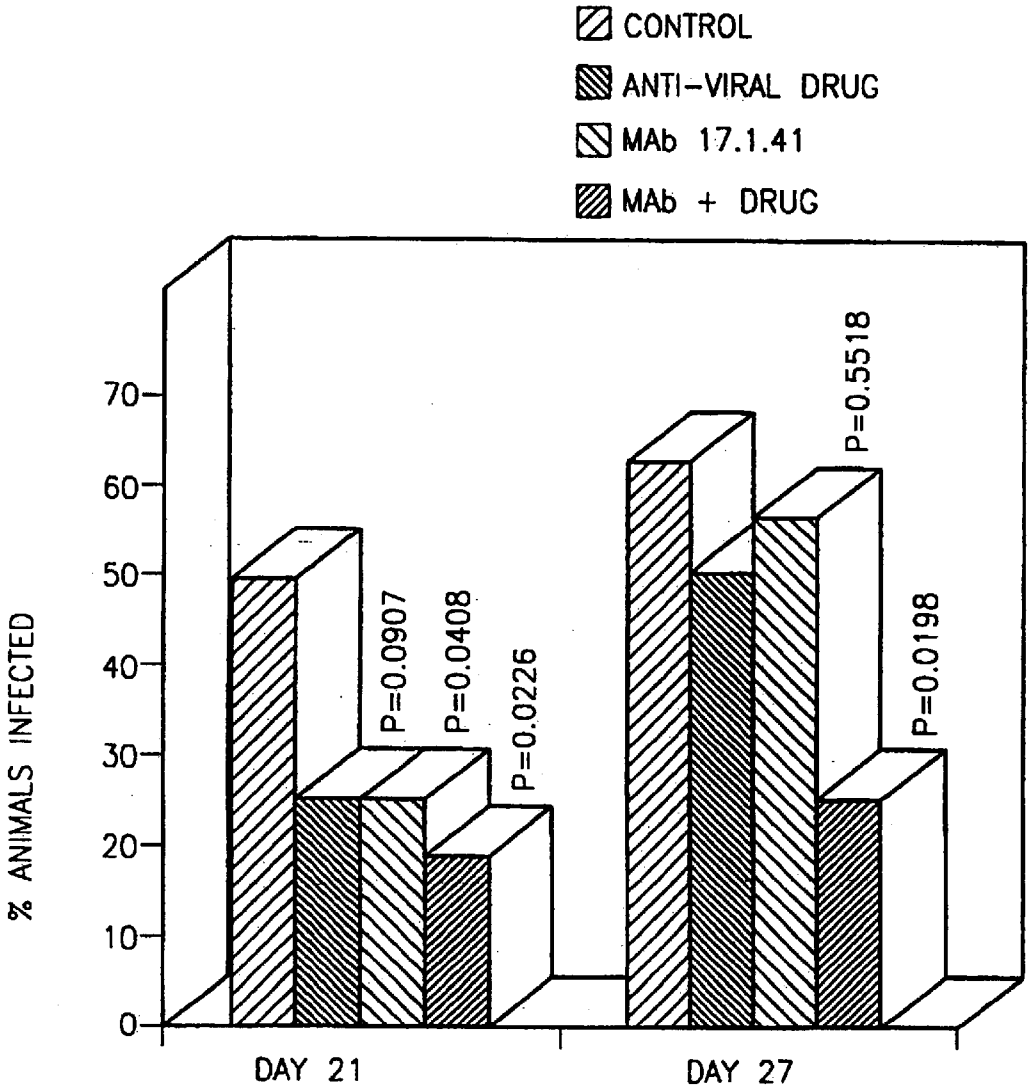

CONTROL
ANTI-VIRAL DRUG
MAb 17.1.41
MAb + DRUG
% ANIMALS INFECTED
70
60
50
40
30
20
10
0
P=0.0907
P=0.0408
P=0.0226
P=0.5518
P=0.0198
DAY 21
DAY 27

FIG.7

		Asp GAT	Ile ATT	Val GTG 9	Met ATG	Thr ACT	Gln CAG 18	Ser TCT	Pro CCA	Leu CTC 27	Ser TCC	Leu CTG	Ser TCC 36	Val GTC	Thr ACC	Pro CCT 45	Gly GGA	Glu GAG	Pro CCG 54
Ala GCC	Ser TCC	Ile ATC 63	Ser TCC	Cys TGC	Arg AGG 72	Ser TCT	Ser AGT	Gln CAG 81	Ser AGC	Leu CTC	Leu CTG 90	His CAT	Arg AGG	Ser TCT 99	Gly GGA	Asn AAC	Asn AAC 108	Tyr TAT	Leu TTG
Asp GAT	Trp TGG	Tyr TAC 123	Leu CTG	Gln CAG	Lys AAG 132	Pro CCA	Gly GGG	His CAC 141	Ser TCT	Pro CCA	Gln CAG 150	Leu CTC	Leu CTG	Ile ATC 159	Tyr TAT	Val GTG	Gly GGT 168	Ser TCT	Asn AAT
Arg CGG	Ala GCC	Ser TCC 183	Gly GGG	Val GTC	Pro CCT 192	Asp GAC	Arg AGG	Phe TTC 201	Ser AGT	Gly GGC	Ser AGT 210	Gly GGA	Ser TCA	Gly GGC 219	Thr ACA	Glu GAG	Tyr TAT 228	Thr ACA	Leu CTG
Lys AAA	Ile ATC	Ser AGT 243	Arg AGA	Val GTG	Glu GAG 252	Ala GCT	Glu GAG	Asp GAT 261	Val GTT	Gly GGG	Val GTA 270	Tyr TAT	Tyr TAC	Cys TGC 279	Met ATG	Gln CAA	Ala GCT 288	Leu CTA	Gln CAA
Thr ACT	Pro CCT	Arg CGG 303	Thr ACT	Phe TTT	Gly GGC 312	Gln CAG	Gly GGG	Thr ACC 321	Lys AAG	Leu CTG	Glu GAG 330	Ile ATC	Lys AAA						

FIG.8

		Gln CAG	Val GTG	Gln CAG 9	Leu CTG	Val GTG	Glu GAG 18	Ser TCA	Gly GGG	Gly GGA 27	Gly GGC	Val GTG	Val GTC 36	Arg CGG	Pro CCT	Gly GGG 45	Arg AGG	Ser TCC	Leu CTG 54	Arg AGA
Leu CTC	Ser TCC	Cys TGT 66	Ala GCA	Ala GCC	Ser TCT 75	Gly GGA	Phe TTC	Ala GCC 84	Phe TTC	Ser AGT	Asp GAC 93	Tyr TAT	Ser AGT	Ile ATA 102	Asn AAC	Trp TGG	Val GTC 111	Arg CGC	Gln CAG	Ala GCT 120
Pro CCA	Gly GGC	Lys AAG 129	Gly GGA	Leu CTG	Glu GAG 138	Trp TGG	Val GTG	Ala GCA 147	Ile ATT	Ile ATT	Ser TCA 156	Tyr TAT	Asp GAT	Gly GGA 165	Arg AGA	Ile ATT	Thr ACA 174	Tyr TAC	Tyr TAT	Arg AGA 183
Asp GAC	Ser TCC	Val GTG 192	Lys AAG	Gly GGC	Arg CGA 201	Phe TTC	Thr ACC	Ile ATC 210	Ser TCC	Arg AGA	Asp GAC 219	Asp GAC	Ser TCC	Lys AAG 228	Asn AAC	Thr ACG	Leu CTG 237	Tyr TAT	Leu CTG	Gln CAA 246
Met ATG	Asn AAC	Ser AGC 255	Leu CTG	Arg AGA	Thr ACT 264	Glu GAG	Asp GAC	Thr ACG 273	Ala GCT	Val GTG	Tyr TAT 282	Tyr TAC	Cys TGC	Ala GCG 291	Arg AGA	Gln CAG	Tyr TAT 300	Tyr TAC	Asp GAT	Phe TTT 309
Trp TGG	Ser AGT	Gly GGT 318	Ser TCT	Ser TCG	Val GTT 327	Gly GGG	Arg CGT	Asn AAC 336	Tyr TAC	Asp GAC	Gly GGC 345	Met ATG	Asp GAC	Val GTC 354	Trp TGG	Gly GGC	Leu CTA 363	Gly GGG	Thr ACC	Thr ACG 372
Val GTC	Thr ACC	Val GTC 381	Ser TCC	Ser TCA																

FIG.9

HUMAN MONOCLONAL ANTIBODY AGAINST HEPATITIS B VIRUS SURFACE ANTIGEN (HBVSAG)

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

FIELD OF THE INVENTION

The present invention concerns a hybridoma cell line producing human antibodies capable of binding to the hepatitis B virus surface antigen, antibodies produced by the cell lines, and various uses thereof.

BACKGROUND OF THE INVENTION

Hepatitis B virus (HBV) infection is a major worldwide health problem. Approximately 5% of the world population is infected by HBV and chronically infected patients carry a high risk of developing cirrhosis and hepatocellular carcinoma. (Progressive Hepatitis Research: Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Hepatitis Delta virus (HDV) Ed. O. Crivelli, Sorina Biomedica, 1991).

The immune response to HBV-encoded antigens includes both a cellular immune response which is active in the elimination of HBV infected cells, as well as a humoral antibody response to viral envelope antigens which contributes to the clearance of circulating virus particles. The dominant cause of viral persistence during HBV infection is the development of a weak antiviral immune response.

Recombinant HBV vaccines provide a safe and effective means for active immunization against HBV, however, they do not always induce a sufficient and rapid antibody response.

Interferon-α has been used in the therapy of Hepatitis B infection showing an efficacy of only 30–40% in highly selected patients.

In addition, passive immunization with human polyclonal anti Hepatitis B antisera has been shown to be effective in delaying and even preventing recurrent HBV infection (Wright, T. L. and Lau, J. Y. N. The Lancet 342:1340–1344, (1993)). Such human polyclonal antisera are prepared from pooled plasma of immunized donors. These preparations are very expensive and available in relatively small amounts. Furthermore, pooled plasma may contain contaminated blood samples and thus treatment with such antisera increases the patient's risk to contract other viral infections such as hepatitis C or HIV.

An alternative approach for the treatment of HBV infections concerns the use of monoclonal antibodies (MoAb).

PCT patent application PCT/NL94/00102 discloses human monoclonal antibodies directed against Hepatitis B surface antigen which are secreted by the hybridoma cell lines Mab 4-7B and Mab 9H9. The monoclonal antibody secreted by the cell line Mab 4-7B recognizes a linear epitope of HBVsAg and is different from the Mab 9H9 monoclonal antibody which recognizes a conformational epitope. The antibodies are claimed for simultaneous use in the treatment of chronic Hepatitis B infections.

PCT patent application PCT/US92/09749 discloses human monoclonal antibodies against HBVsAg which are secreted by the hybridoma cell lines PE1-1, ZM1-1, ZM1-2, MD3-4 and LO3-3. The antibodies bind to different HBV epitopes and are used for reducing the level of circulating HBVsAg.

Japanese Patent Application JP 93066104 discloses a hybridoma of a human lymphocyte cell strain TAW-925 and a human lymphocyte transformed by Epstein-Barr virus. The hybridoma produces a human monoclonal antibody against HBVsAg.

U.S. patent application Ser. No. 4,883,752 discloses preparation of human-derived monoclonal antibody to HBVsAg, by administration of HBVsAg vaccine to humans, recovering their lymphocytes, stimulating the lymphocytes in vitro by a non specific stimulator, fusing said cells with a myeloma cell, and selecting for hybridomas with secrete and HBVsAg antibodies.

Ichimori et al., Biochem. and Biophysic. Research Communications 129(1):26–33, 1985 discloses a hybridoma secreting human anti HBVsAg monoclonal antibodies which recognize the a-determinant of HBVsAg. Later, Ichimori, et al., supra 142(3):805–812, 1987 disclosed another hybridoma which stably secretes human monoclonal antibody against HBsAg.

SUMMARY OF THE INVENTION

In accordance with the present invention, a hybridoma cell line is provided which secretes human antibodies capable of binding to the Hepatitis B surface antigen (HBVsAg).

In accordance with the invention, peripheral blood lymphocytes (PBL) were obtained from human individuals having a high titer of anti HBVsAg antibodies. Such individuals may either have been previously infected with HBV, actively immunized with HBV antigens or spontaneously showing a high level of such antibodies. A most preferred human donor is an individual which tested negative for the presence of HBV but shows a high titer of antibodies against HBVsAg. PBLs from the human donor may be obtained either by whole blood donation or by leukophoresis.

The human PBLs are then activated in vitro by their incubation with pokeweed mitogen (PWM). After activation the PBLs are fused in vitro preferably with a human-mouse fusion partner such as a heteromyeloma by techniques well known in the art (e.g. Kohler & Milstein, Nature, 256:495–497, 1975). The generated hybridoma cell lines are either cultured in vitro in a suitable medium wherein the desired monoclonal antibody is recovered from the supernatant or, alternatively the hybridoma cell lines may be injected intraperitoneally into mice and the antibodies harvested from the malignant ascitis or serum of these mice. The supernatant of the hybridoma cell lines are first screened for production of human IgG antibodies by any of the methods known in the art such as enzyme linked immunosorbent assay (ELISA) or radioimmuno assay (RIA). Hybridomas testing positive for human IgG are then further screened for production of anti HBVsAg antibodies by their capability to bind to HBVsAg.

In accordance with the preferred embodiment of the present invention, a hybridoma cell line designated herein as "17.1.41" which was deposited on May 22, 1996, at the European Collection of Cell Cultures (ECACC, CAMR, Salisbury, Wiltshire, SP40JG, U.K.) under the Accession No. 96052169 is provided. Anti HBVsAg human monoclonal antibodies secreted by the above hybridoma cell line designated herein as "Ab17.1.41" as well as fragments thereof retaining the antigen binding characteristics of the antibodies and antibodies capable of binding to the antigenic epitope bound by Ab17.1.41 are also provided. Such fragments may be, for example, Fab or $F(ab)_2$ fragments obtained by digestion of the whole antibody with various

enzymes as known and described extensively in the art. The antigenic characteristics of an antibody are determined by testing the binding of an antibody to a certain antigenic determinant using standard assays such as RIA, ELISA or FACS analysis.

The antibodies of the invention have a relatively high affinity to HBVsAg being in the range of about 10^{-9}M to about 10^{-10}M as determined by a competitive ELISA assay.

The antigen bound by the antibodies defined above also constitutes an aspect of the invention.

Further aspects of the present invention are various diagnostic prophylactic and therapeutic uses of the Ab 17.1.41 monoclonal antibodies and the Ag bound by these antibodies. In accordance with this aspect of the invention, pharmaceutical compositions comprising the Ab17.1.41 antibodies may be used for the treatment of chronic Hepatitis B patients by administering to such a patient a therapeutically effective amount of the antibodies or fragments thereof capable of binding to the HBVsAg being an amount effective in alleviating the symptoms of the HBV infection or reducing the number of circulating viral particles in an individual.

In addition to the antibodies of the invention the pharmaceutical compositions may optionally also comprise a carrier selected from any of the carriers known in the art. One example of such a carrier is a liposome. The pharmaceutical compositions of the invention may also comprise various diluents and adjuvants known per se.

The compositions of the invention may be administered by a variety of administration modes including parenterally, orally etc. Compositions comprising the antibodies of the invention, as described above, may be administered in combination with other anti viral agents. Such agents may include, as a non limiting example: Interferons, anti HB monoclonal antibodies, anti HB polyclonal antibodies, nucleoside analogs, and inhibitors of DNA polymerase. In the case of such a combination therapy the antibodies may be given simultaneously with the anti viral agent or sequentially either before or after treatment with the anti viral agent.

Such pharmaceutical compositions may also be used, for example, for immunization of new born babies against HBV infections or for immunization [cf] *of* liver transplantation patients to eliminate possible recurrent HBV infections in such patients.

By a further embodiment, the antibodies of the invention may also be used in a method for the diagnosis of HBV infections in an individual by obtaining a body fluid sample from the tested individual which may be a blood sample, a lymph sample or any other body fluid sample and contacting the body fluid sample with a human anti HBVsAg antibody of the invention under conditions enabling the formation of antibody-antigen complexes. The level of such complexes is then determined by methods known in the art, a level significantly higher than that formed in a control sample indicating an HV infection in the tested individual. In the same manner, the specific antigen bound by the antibodies of the invention may also be used for diagnosis of HB infection in an individual by contacting a body fluid sample from the tested individual with the antigen as described above and determining the formation of antigen Ab in the sample.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a photograph showing Hepatitis B infected liver sections stained with the anti HBVs antibodies of the invention. All sections were stained with a "secondary" antibody, i.e. goat anti human or anti mouse Ig conjugated to biotin.

A—negative control. No first antibody.

B—positive control. First antibody—mouse anti HB antibody and a secondary anti-mouse Ig.

C—staining with anti HBVsAg Ab 17.1.41.

Reference will now be made to the following Examples which are provided by way of illustration and are not intended to be limiting to the present invention.

FIG. **2** is a schematic representation of the binding of Ab17.1.41 to a set of well characterized HBsAg types. The y axis represents optical density units. The x axis represents different HBsAg types.

FIG. **3** is a graphic representation of hepatitis B viremia score, as defined in example 3. Each dot in the graph represents one animal.

FIG. **4** is a graphic representation of the percentage of HBV infected animals at days 18 and 25 in the untreated group and Ab17.1.41 treated group (in the treatment model).

FIG. **5** is a graphic representation of the percentage of HBV infected animals at days 10 and 17 in the untreated group and Ab17.1.41 treated group (in the combined prophylaxis/inhibition model).

FIG. **6** is a graphic representation of the percentage of HBV infected animals at days 11 and 18 in the untreated group and Ab 17.1.41 treated group (in the combined inhibition/treatment model).

FIG. **7** is a graphic representation of the percentage of HBV infected animals at days 21 and 27 in the untreated group (control), the group treated with an anti viral drug, the group treated with Ab17.1.41 and the group treated with both the anti viral drug and Ab17.1.41 (Mab+Drug).

FIG. **8** Nucleic acid sequence (SEQ ID NO:1) and corresponding amino acid sequence (SEQ ID NO:2) of the light chain of the variable domain of Ab17.1.4 1.

FIG. **9** Nucleic acid sequence (SEQ ID NO:3) and corresponding amino acid sequence (SEQ ID NO:4) of the heavy chain of the variable domain of Ab 17.1.41.

EXAMPLES

Materials and Methods

In vitro Activation:

Peripheral blood lymphocytes (PBL) were obtained after informed consent by leukophoresis from donors positive for HBs antibodies and negative for HBV. PBLs were washed twice, counted and resuspended in PBS to the desired cell concentration. PBL were separated from granulocytes and erythrocytes on a Ficoll-hypaque gradient (UNI-SEP maxi; Eldan Tech., Jerusalem, Israel) and subsequently stimulated for 3–4 days with pokeweed mitogen (PWM; Gibco BRL, Life Technologies Inc., Grand Island, N.Y.) diluted 1:100 and with Antigen at 200 ng/ml in RPMI-1640 medium with 10% (v/v) fetal calf serum (FCS) supplemented with 10 U/ml penicillin, 10 μg/ml streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate, 1% (v/v) non-essential amino acids (Biological Industries, Belt Haemek, Israel) and 10^{-4} M 2-mercaptoethanol (Sigma, St. Louis) (Complete Medium).

Cell Fusion:

Cells were mixed with the human-mouse heteromyeloma HMMA2.1 1TG/0 (Posner et al.) at 3:1 ratio. Fusion was performed with 50% (w/v) PEG 1500 (Boehringer Manheim GmbH) in a standard procedure. Fused cells were seeded at a concentration of 30000 cells/well in 96-well U-bottom microtiter plates (Nunc, Denmark) in complete medium containing HAT-supplement (1×) (Biological Industries, Beit Haemek, Israel). Cells were fed with fresh HAT-medium a week latter. Two weeks after fusion supernatants were harvested for ELISA and medium was replaced with fresh HT-medium.

Hybridoma cultures secreting specific anti-HBs Ig were cloned at 0.5 cell/well in 96-well U-bottom microtiter plates.

Determination of Human Immunoglobulin:

Sera were tested for antigen specific and total human ig. Total human Ig was quantified by sandwich ELISA using goat $F(ab)_2$-purified anti-human IgG+IgM+IgA (Zymed Laboratories, San Francisco, Calif.) as the capture agent and peroxidase-conjugated purified goat anti-human (Zymed Laboratories) as the detection reagent. Human serum of known immunoglobulin concentration was used as the standard (Sigma, Rehovot, Israel). Microplates (Nunc, Roskilde, Denmark) pre-coated with the capture reagent (2.5 ug/ml, 50 ul/well) and blocked with 1% BSA were incubated overnight at 4C with dilutions of plasma from 1:20000 to 1:640000, or the standard from 0.2 to 0.06 ug/ml, then washed 5 times with PBS-Tween solution. The detection reagent was added and the plates were incubated for 1 h at 37C, then washed again 3 times. Fresh substrate solution (TMB, Sigma) was added and, after peroxidase-catalyzed color development, the reaction was stopped by addition of 10% sulfuric acid. Absorbance at 450 nm was quantified on an ELISA reader (Dynatech, Port Guernsey, Channel Islands, UK).

Concentration of antigen-specific human antibodies in mice sera was determined by HBsAb EIA kit (ZER, Jerusalem, Israel).

Human antibodies in hybridoma supernatants were determined by overnight incubation of supernatants on goat anti-human IgG+A+M (Zymed) coated plates, with goat anti-human IgG-peroxidase conjugated as the secondary reagent.

Antigen-specific antibodies in hybridoma supernatants were determined as above using Hbs antigen coated plates.

Determination of Human IgG Subclasses:

Human IgG subclasses were determined by sandwich ELISA using goat $F(ab)_2$-purified anti-human IgG+IgM+IgA (Zymed Laboratories, San Francisco, Calif.) coated plates and Hbs antigen coated plates. Mouse anti-human IgG subclasses (Sigma) were used as second antibody and peroxidase-conjugated purified goat anti-human (Zymed Laboratories) as the detection reagent.

Statistic Analysis:

Statistical analysis was performed using the Stat View II program (Abacus Concepts, Inc., Berkeley, Calif.) on a Mackintosh Quadra 605 or Microsoft Excel 5.0 (Microsoft) on a 486 DX2 PC compatible. Student t-test, Anova correlation and regression analysis were utilized to calculate probability (p) and correlation coefficient (r) values. Results are presented as mean ± standard error.

Affinity Constant Measurements:

Determination of affinity constants (K_D) of the different anti-HBs antibodies to ad antigen (Chemicon Cat. No. AG 850) in solution were performed according to Friguet et al. (Journal of Immunological Methods, 77:305–319, 1985). The antigen at various concentrations (3.5×10^{-10}M to 1.4×10^{-9}M) was first incubated in solution with a constant amount of antibody (3.4×10^{-11}M), in 0.1 M sodium phosphate buffer containing 2 mM EDTA and 10 mg/ml BSA, pH 7.8 (medium buffer). After o.n. incubation at 20 C the concentration of free antibody was determined by an indirect ELISA. A volume of 300 ul of each mixture were transferred and incubated for 2 h at 20 C into the wells of a microtitration plate (Nunc) previously coated with Ad (50 µl/well at 1 µg/ml in 0.1 M $NaHCO_3$ buffer, pH 9.6 for 2 h at 37° C.). After washing with PBS containing 0.04% Tween 20, the bound antibodies were detected by adding HRP-$F(ab')_2$ Goat anti human IgG (Zymed) diluted 1:3000 with medium buffer, 50 µl/well 2 h at 20° C. The plate was developed with TMB chromogen (Sigma T-3405 tablets) 50 µl/well, the reaction stopped with 10% H_2SO_4 50 µl/well and the plate read in an ELISA reader at 450 nm. The conditions were chosen so that the resulting f values (see Friguet et al.) were around 0.1. The antibody concentration used was deduced from an ELISA calibration done on the same plate. The affinity constant KD was calculated from the relevant Scatchard plot.

Inhibition Assays:

The inhibition assay was performed in microtiter plates coated with HBs particles (2 µg/ml in PBS). The plate was blocked with 3% BSA in PBS. Hybridoma supernatants containing anti HBs antibodies were serially diluted. 50 µl of each dilution were added to the coated microtiter wells. Subsequently, 50 µl of HBs particles (ad/ay, 0.5 µl/ml in PBS) or PBS alone were added to each well. The plates were incubated overnight at room temperature in a humid chamber and washed 5 times with PBS-Tween. Next, 50 µl of goat anti human IgG conjugated to HRP (diluted 1:5000 in PBS) were added to each well. After a 4 hour incubation at room temperature in a humid chamber the plates were washed 5 times with PBS-Tween, and TMB was added to each well. Results were read using an ELISA reader, in a wavelength of 450 nm.

Immunohistostaining:

HBV positive liver fragment was fixed in 4% neutral buffered formaldehyde for 24 h and then embedded in paraffin using routine procedures. Section of 4 µm thickness were cut from paraffin blocks and mounted on polylysine-coated slides. After deparaffinization and peroxidase quenching staining was performed using our monoclonal Human anti-HBs Protein A-purified antibodies followed by biotinylated Goat anti-Human IgG (H+L) (Zymed, San Francisco, Calif.) using Histostain-SPTM kit (Zymed) according to the manufacture's recommendation. Control slides without using the 1 st Human anti-HBs antibody were stained in parallel.

Sequence analysis:

Total RNA was isolated from 10×10^6 hybridoma cells with RNAsol B reagent (TEL-TEX, Inc. Friendswood, Tex.). cDNA was prepared from 10 µg of total RNA with reverse transcriptase and oligo dT (Promega, Madison, Wis.) according to standard procedures. PCR was performed on 1/50 of the RT reaction mixture with V_H, V_λ or V_K5' leader primers and 3' primers corresponding to human constant region. The PCR fragments were cloned into pGEM-T vector (Promega). The inserts were sequenced using an ABI 377 sequencing machine. Sequences were analyzed by comparison to Genbank and by alignment to Kabat sequences (Kabat et al. 1991, Sequences of proteins of immunological interest (5^{th} Ed.) U.S. Dept. of Health and Human Services, National Institutes of Health, Bethesda, Md.).

Example 1

Human peripheral blood lymphocytes (PBL) from donors positive for anti HBVs antibodies were obtained and activated in vitro with PWM as described above. The cells were then fused with a human mouse heteromyeloto form hybridoma cell lines. One stable hybridoma clone secreting specific human anti HBVsAg designated 17.1.41 was characterized. The antibodies secreted by the above clone were purified on a protein A column as well as on an anti human Ig-agarose column and were found to be of the IgG1 Vκ type. The affinity constant of the antibodies to HBVsAg was 1.34×10^{-9}. Specificity was tested by competitive inhibition assay using HBV surface antigen of the ad-ay (1:1).

Example 2

The 17.1.41 antibodies were used for staining human liver fragments as described above. As seen in FIG. **1**, the 17.1.41 antibodies were able to detect HBV particles present in the infected liver fragments.

The gene encoding the variable region of Ab 17.1.41 was isolated, fully sequenced, and its subgroups and CDRs were determined.

The antibody has a fully human Ig gene sequence as determined by alignment to Genebank sequences and Kabat protein sequences. FIG. **8** shows the nucleotide sequence of the cDNA encoding the light chain of the variable region of Ab 17.1.41 and its corresponding amino acid sequence (Sequence identification nos. I and 3). FIG. **9** shows the nucleotide sequence of the cDNA encoding the heavy chain of the variable region of Ab17.1.41 and its corresponding amino acid sequence (Sequence identification nos. 2 and 4).

The sequencing data revealed that the variable region of Ab 17.1.41 consists of the subgroups V_{H3}, J_H6, V_{K2} and J_{K2}.

HBV genomes are classified into six groups A to F, based on the degree of similarity in their nucleotide sequences. The genetic variability of HBV is further reflected in the occurrence of different serotypes of HBsAg. The common determinant 'a' and two pairs of mutually exclusive determinants 'd/y' and 'w/r' enable the distinction of four major subtypes of HBsAg: adw, adr, ayw and ayr. Additional determinants designated subdeterminants of w(w1 to w4) have allowed the definition of four serotypes of ayw (ayw1–4) and two serotypes of adw, i.e. adw2 and adw4. Additional subtype variation is added by the q determinant, which is present on almost all subtypes. Its absence is marked by a 'q-' sign. The kind of HBV serotypes recognized by Ab 17.1.41 was examined using a set of 15 different HBsAg types (Norder et al., 1992, Journal of General Virology, 73, 3141; Magnius and Norder, 1995, Intervirology, 38, 24–34). As can be seen in FIG. **2**. Ab 17.1.41 has a broad reactivity towards all tested subtypes and genotypes, except for C adw2.

Example 3

The biological activity of Ab 17.1.41 was characterized using the following HBV animal model: a mouse was treated so as to allow the stable engraftment of human liver fragments. The treatment included intensive irradiation followed by transplantation of scid (severe combined immunodeficient) mice bone marrow. Viral infection of human liver fragments was performed ex-vivo using HBV positive human serum (EP 699 235).

The animal model was used in three different modes representing various potential uses of the antibodies: treatment mode, combined prophylaxis/inhibition mode and combined inhibition/treatment.

1. Treatment mode—This model demonstrates the ability to use the antibody to treat chronic HBV infection. Mice were transplanted with HBV infected human liver fragments. The mice were treated with Ab 17.1.41 at days 16, and 17 post liver transplantation. HBV DNA was tested on days 18 and 25. The number of HBV DNA copies (the viral load) in mouse sera was determined using PCR. We use the term "viremia score" as a mathematical representation of the viral load. The viremia score was determined as follows:

Viremia score	viral load = HBV DNA copies/ml serum
0	viral load $< 5 \times 10^3$
1	$5 \times 10^3 <$ viral load $< 5 \times 10^4$
2	$5 \times 10^4 <$ viral load $< 5 \times 10^5$
3	viral load $> 5 \times 10^5$

As can be seen in FIG. **3**, there is a significant reduction in the viremia score in the group treated with the antibody. In addition, as can be seen in FIG. **4**, the percentage of infected animals in the treated group are significantly lower (very low p values) as compared to the untreated group.

2. Combined prophylaxis/inhibition mode—This model represents liver transplantation. In this model mice were treated with Ab 17.1.41 (10 I.U./mouse) three days before liver transplantation followed by transplantation of human liver fragments which were ex vivo infected with HBV in the presence of Ab 17.1.41 (100 I.U.). HBV DNA was tested in mice sera 10 and 17 days after transplantation. As can be seen in FIG. **5**, there was a significant reduction in the percentage of infected animals in the treated group compared to the control group.
3. Combined inhibition/treatment mode—a) HBV positive human serum was preincubated with Ab 17.1.41 followed by standard ex vivo liver infection. b) Mice were treated with Ab 17.1.41 at days 0 and 7 post transplantation. HBV DNA in mice sera was tested on days 11 and 18. As can be seen in FIG. **6**, the percentage of infected animals in the Ab 17.1.41 treated group was significantly reduced but rebounded about two weeks after the treatment was stopped.

Example 4

In the following experiment we tested the possibility to use 17.1.41 in combination with another anti viral agent in the HBV model described above. Mice were treated with the anti viral drug (a nucleoside analogue, 0.5 mg/mouse/day) at days 17–20 post transplantation. A group of mice was further treated with Ab 17.1.41 at days 19 and 20. The presence of HBV DNA in mice sera was tested on days 21 and 27. As can be seen in FIG. **7**, immediately after treatment either with the anti viral drug or with our monoclonal antibody there was a marked reduction in the number of animals infected. However, viral load rebounded in each group that was treated with one individual drug. Only the group that was treated with the combination of the anti viral drug and Ab 17.1.41 did not show an increase in the number of animals infected.

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Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Tyr Thr Leu Lys Ile
 65                  70                  75                  80

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Ser Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
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 65                  70                  75                  80

ctg caa atg aac agc ctg aga act gag gac acg gct gtg tat tac tgc      288
Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Ala Val Tyr Tyr Cys
                 85                  90                  95

gcg aga cag tat tac gat ttt tgg agt ggt tct tcg gtt ggg cgt aac      336
Ala Arg Gln Tyr Tyr Asp Phe Trp Ser Gly Ser Ser Val Gly Arg Asn
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         35                  40                  45

Ala Ile Ile Ser Tyr Asp Gly Arg Ile Thr Tyr Tyr Arg Asp Ser Val
     50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Leu Tyr
 65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Ala Val Tyr Tyr Cys
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Ala Arg Gln Tyr Tyr Asp Phe Trp Ser Gly Ser Ser Val Gly Arg Asn
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Tyr Asp Gly Met Asp Val Trp Gly Leu Gly Thr Thr Val Thr Val Ser
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Ser
```

What is claimed is:

1. A human monoclonal antibody Ab17.141, which is secreted by the European Collection of hybridoma cell line deposited in the Cell Cultures (ECACC) under Accession No. 96052169, *or a fragment thereof which retains the antigen binding characteristics of Ab17.1.41*.

2. The hybridoma cell line deposited at the ECACC on May 22, 1996 under Accession No. 96052169.

3. A pharmaceutical composition for the treatment of Hepatitis B Virus (HBV) infections comprising as an active ingredient an antibody in accordance with claim **1** together with a pharmaceutically acceptable carrier.

4. A method for the treatment of Hepatitis B Virus (HBV) infections comprising administering to an individual in need a therapeutically effective amount of antibodies according to claim **1**.

5. A method for reducing the occurrence of Hepatitis B virus (HBV) infections in a population of individuals, comprising administering a human monoclonal antibody Ab 17.1.41 or a fragment thereof which retains the antigen binding characteristics of Ab 17.1.41 in accordance with claim **1** to a population of individuals to reduce the occurrence of HBV infections in the population.

6. A pharmaceutical composition for the treatment of Hepatitis B Virus infections comprising as an active ingredient an antibody in accordance with claim **1** adopted for use in combination with at least one other active ingredient being an anti viral agent.

7. A pharmaceutical composition according to claim **6** wherein the anti viral agent is selected from the group consisting of: interferons, anti-Hepatitis B (HB) monoclonal antibodies, anti HB polyclonal antibodies, nucleoside analogues and inhibitors of DNA polymerase.

8. A pharmaceutical composition according to claim **6** wherein the anti viral agent is a nucleoside analogue.

9. A method for the treatment of HBV infections comprising administering to an individual in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim **6**.

10. A method for the treatment of HBV infections comprising administering to an individual in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim **7**.

11. A method for the treatment of HBV infections comprising administering to an individual in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim **8**.

12. A method for reducing the occurrence of HBV infections according to claim 5, wherein the population of individuals include liver transplantation patients.

* * * * *