Hepatitis B virus specific human antibodies

The invention relates to binding agents, preferably to human, humanized or otherwise sequence optimized antibody fragments, which are specifically binding to Hepatitis B virus (HBV) preS1 antigen. These binding agents of the invention recognize an epitope of the preS1 antigen which has not yet been described in the art and have unique properties. These binding agents disclosed herein are binding to HBV subviral particles and are furthermore capable of neutralizing HBV, i.e. they inhibit the replication of HBV. The invention further relates to the use of these binding agents for the treatment and/or prophylaxis of HBV infection.
Hepatitis B Virus Specific Human Antibodies

FIELD OF THE INVENTION

[0001] The invention relates to binding agents, preferably to human, humanized or otherwise sequence optimized antibody fragments, which are specifically binding to Hepatitis B virus (HBV) preS1 antigen. These binding agents of the invention recognize an epitope of the preS1 antigen which has not yet been described in the art and have unique properties. These binding agents disclosed herein are binding to HBV subviral particles and are furthermore capable of neutralizing HBV, i.e. they inhibit the replication of HBV. The invention further relates to the use of these binding agents for the treatment and/or prophylaxis of HBV infection.

RELATED ART

[0002] Hepatitis B virus (HBV) is an enveloped RNA virus that infects human liver and replicates via reverse transcription of the pregenomic RNA. Infected patients develop acute hepatitis, which may develop into chronic hepatitis with high risk of liver cirrhosis and primary liver carcinoma. Different types of vaccines with distinct subunit compositions have been developed (reviewed in: Shouval D. (2003), J. Hepatol. 39(Suppl. 1):70-76). However, about 10 % of the population respond poorly or not at all to current HBV vaccines and remain unprotected. Post exposure prophylactic treatment is of great importance to reduce the risk of infection. Current post-exposure protection includes the use of human HBV-specific immunoglobulins which are made from human plasma. Despite extensive testing, a residual risk of the transmission of infectious agents by these products can never be completely excluded. A completely safe post-exposure prophylactic treatment would consist of a recombinantly produced, HBV-neutralizing human monoclonal antibody.

[0003] The envelope of human HBV contains three co-terminal proteins, designated small (S), middle (M), and large (L) surface protein. The S protein is the most abundant of the three and consists of 226 amino acids. The M protein consists of the S protein sequence and an additional 55 amino acids at the N-terminus, designated the preS2 sequence. The L protein consists of the S and preS2 sequences and contains an additional 119 (or 108, depending on the HBV subtype) amino acids at the N-terminus, designated the preS1 sequence. Antibodies against the S protein have been shown to protect against infection (Waters et al. (1992) Virus Res. 22:1-12). In addition, while the preS2 region appears to be nonessential for virus
attachment (Fernholz et al. (1993) Virology 194:137-148), attachment of HBV to human hepatocytes is mediated by the preS1 domain (Neurath et al. (1986) Cell 46:429-436), and rabbit polyclonal or mouse monoclonal antibodies against preS1 have been show to neutralize HBV by blocking cell binding (Neurath et al. (1989) Vaccine 7: 234-236; Petit et al. (1991) Mol. Immunol. 28:517-521; Pontissio et al. (1989) Virology 173:522-530). However, none of these antibodies is therapeutically useful due to their strong immunogenicity in humans.

**SUMMARY OF THE INVENTION**

[0004] The invention provides binding agents, preferably human, humanized or otherwise sequence optimized antibody fragments, which are specifically binding Hepatitis B Virus (HBV) preS1 antigen. It has surprisingly been found that these binding agents of the invention recognize a novel epitope of preS1 antigen which is comprised by the peptide TANPDWDFNP (SEQ ID NO:32) and furthermore there binding agents have unique properties. The sequence X₁X₂PDWX₃X₄X₅ (SEQ ID NO:34) with X₁ to X₅ being any amino acid residue was identified as the minimal epitope recognized by the binding agents of the invention. This sequence is common to the preS1 antigen of a broad spectrum of HBV genotypes, including the genotypes A, A (afr), B, C, C (aus), D, E, F, G and H. An inventive binding agent showed cross-reactivity with the preS1 consensus sequence (SEQ ID NO:18) and with characteristic preS1 peptides derived from each these genotypes, wherein the peptides corresponding to the 4 major HBV genotypes A-D were recognized equally well. A binding agent of the invention bound a GST-preS1 (21-47) fusion protein in a monovalent interaction with a Kd value of about 100 pM. It has also been demonstrated that a binding agent of the invention binds to HBV genotype D subviral particles with an EC50 value of 12.2 ng/ml (112 pM). This finding demonstrates that a binding agent of the invention is capable of specifically binding to preS1 in the context of the native surface protein of HBV which is comprising the preS1 and preS2 regions. Finally, it was demonstrated that a binding agent of the invention showed potent neutralizing activity towards HBV, i.e. it was capable of inhibiting the replication of HBV. The EC50 value for the neutralizing activity was consistently found to be below 1 nM.

[0005] In its main aspect, the invention provides a binding agent specifically binding Hepatitis B Virus (HBV) preS1 antigen e.g. the novel epitope as described herein, wherein said binding agent comprises an antibody that is not able to bind to or only able to bind in a reduced manner (such as e.g. about 50% or less, more preferably 40% or less, more preferably
30%, more preferably 20%, and even more preferably only 10% average binding compared to the relevant wildtype receptor such as e.g. Fc receptor measured e.g. by a competition assay) to various cell receptors (e.g. at least one such receptor) it normally binds such as Fc receptors. In a preferred aspect, the invention provides a binding agent specifically binding Hepatitis B Virus (HBV) preS1 antigen, e.g. the novel epitope as described herein, wherein said binding agent comprises an antibody that is not able to bind to or only able to bind in a reduced manner to Fcey receptors (R) such as FcgR (such as e.g. about 50% or less, more preferably 40% or less, more preferably 30%, more preferably 20%, and even more preferably only 10% average binding compared to FcgR measured e.g. by a competition assay) (see Kathryn et al. 1999, Eur. J. Immunol. 29: 2613-2624).

[0006] In another main aspect, the invention provides a binding agent specifically binding Hepatitis B Virus (HBV) preS1 antigen, e.g. the novel epitope as described herein, wherein said binding agent comprises an antibody that has a flexible hinge region directly adjacent to the antigen binding site, such as in a single chain antibody Fc fusion protein, unlike in an IgG such as e.g. an IgG1, where the hinge region is not directly adjacent to the antigen binding site but separated from it by the CH1 domain.

[0007] In another main aspect, the invention provides a binding agent specifically binding Hepatitis B Virus (HBV) preS1 antigen, e.g. the novel epitope as described herein, wherein said binding agent comprises an antibody that is capable of specifically binding the novel epitope of preS1 antigen which is comprised by the peptide TANPDWDFNP (SEQ ID NO:32), more preferably said binding agent comprises an antibody that is capable of specifically binding the novel minimal epitope of preS1 antigen which is comprised by the peptide X1X2PDWDX3X4X5 (SEQ ID NO:34) with X1 to X5 being any amino acid residue. In a preferred aspect, the invention provides a binding agent specifically binding Hepatitis B Virus (HBV) preS1 antigen, wherein said binding agent comprises an antibody that a) is either not able to bind to or only able to bind in a reduced manner to various cell receptors it normally binds such as Fc receptors (e.g. to FcgR) and/or b) has a flexible hinge region directly adjacent to the antigen binding site, such as in a single chain antibody Fc fusion protein, unlike in an IgG such as e.g. an IgG1, where the hinge region is not directly adjacent to the antigen binding site but separated from it by the CH1 domain; and wherein said antibody has at least one antigen binding site, wherein said antigen binding site comprises: (a) a HCVR, wherein said HCVR comprises a HC CDR1, a HC CDR2 and a HC CDR3; and (b) a LCVR, wherein said LCVR comprises a LC CDR1, a LC CDR2 and a LC CDR3; wherein
the CDRs of said at least one antigen binding site, when taken as a whole, differ from the
CDRs of an antigen binding site comprising: (a) a HCVR, wherein said HCVR comprises the
HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID
NO:17; and (b) a LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID NO:12, the
LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14; in at most 3, preferably in
at most 2, and most preferably in at most 1 amino acid residue(s).

[0008] In another main aspect, the invention provides a binding agent specifically binding
Hepatitis B Virus (HBV) preS1 antigen, e.g. the novel epitope as described herein, wherein
said binding agent comprises or essentially consists of a scFv or scFc-Fc (muted as described
herein) that has at least one antigen binding site, wherein said antigen binding site comprises:
(a) a HCVR, wherein said HCVR comprises a HC CDR1, a HC CDR2 and a HC CDR3; and
(b) a LCVR, wherein said LCVR comprises a LC CDR1, a LC CDR2 and a LC CDR3;
wherein the CDRs of said at least one antigen binding site, when taken as a whole, differ from
the CDRs of an antigen binding site comprising: (a) a HCVR, wherein said HCVR comprises
the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of
SEQ ID NO:17; and (b) a LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID
NO:12, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14; in at most 3,
preferably in at most 2, and most preferably in at most 1 amino acid residue(s).

[0009] In another main aspect, the invention provides a binding agent specifically binding
Hepatitis B Virus (HBV) preS1 antigen, e.g. the novel epitope as described herein, wherein
said binding agent comprises or essentially consists of an antibody that has at least one
antigen binding site, wherein said antigen binding site comprises: (a) a HCVR, wherein said
HCVR comprises a HC CDR1, a HC CDR2 and a HC CDR3; and (b) a LCVR, wherein said
LCVR comprises a LC CDR1, a LC CDR2 and a LC CDR3; wherein the CDRs of said at
least one antigen binding site, when taken as a whole, differ from the CDRs of an antigen
binding site comprising: (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ
ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and (b) a
LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID NO:12, the LC CDR2 of
SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14; in at most 3, preferably in at most 2,
and most preferably in at most 1 amino acid residue(s).

[0010] In a further preferred aspect, the invention provides a binding agent specifically
binding Hepatitis B Virus (HBV) preS1 antigen, e.g. the novel epitope as described herein,
wherein said binding agent comprises an antibody that a) is either not able to bind to or only
able to bind in a reduced manner to various cell receptors it normally binds such as Fc receptors (e.g. to FcgR) and/or b) has a flexible hinge region directly adjacent to the antigen binding site, such as in a single chain antibody Fc fusion protein, unlike in an IgG such as e.g. an IgG1, where the hinge region is not directly adjacent to the antigen binding site but separated from it by the CH1 domain, and wherein said binding agent comprises an antibody that is cross-blocked or cross blocking the antibody with an antigen binding site that comprises: (a) a HCVR, wherein said HCVR comprises a HC CDR1, a HC CDR2 and a HC CDR3; and (b) a LCVR, wherein said LCVR comprises a LC CDR1, a LC CDR2 and a LC CDR3; wherein the CDRs of said at least one antigen binding site, when taken as a whole, differ from the CDRs of an antigen binding site comprising: (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and (b) a LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID NO:12, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14; in at most 3, preferably in at most 2, and most preferably in at most 1 amino acid residue(s).

[0011] Further aspects of the invention are disclosed herein below.

DESCRIPTION OF THE FIGURES

[0012] Figure 1. Characterization of binding specificity of antibody 2D028. Reactivity of scFv-2D028-Fc with the indicated solid phase-bound 27-mer preS1 sequences was measured at the indicated concentrations.

[0013] Figure 2. Epitope mapping. (A) Sequences of overlapping peptides used for the preparation of GST fusion proteins. (B) Epitope mapping using GST-preS1 fusion proteins. Reactivity of serial dilutions of scFv-2D028-Fc with the indicated proteins was analyzed by ELISA.

[0014] Figure 3. Epitope mapping. (A) Epitope scanning analysis. Reactivity of scFv-2D028-Fc with the indicated solid phase-bound 3- to 10-mer sequences was determined. (B) Point mutation analysis. Binding of scFv-2D028-Fc to all possible solid phase-bound single positional variants of TANPDWDFNP was measured. Each position of the peptide was probed with all 20 possible amino acids, in alphabetical order (A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y). Black bars, reactivity with peptides corresponding to the wild-type sequence.
[0015] **Figure 4.** Neutralization assay. HBV was pre-incubated with different amounts of antibody and used to infect primary *Tupaia belangeri* hepatocytes. Concentrations indicated are final antibody concentrations in the culture medium. Supernatants were harvested and HBsAg concentrations determined by ELISA on the indicated days.

[0016] **Figure 5.** Sandwich ELISA showing simultaneous binding of MA18/7 and 2D028 to preS1(21-47). mAb MA18/7 was coated to the plate and incubated with preS1(21-47) peptide. MA18/7-peptide complexes were then incubated with serial dilutions of the indicated scFv-Fc antibodies. E003, irrelevant control scFv-Fc antibody.

[0017] **Figure 6.** Sequence of mature scFv-Fc "mut" (signal peptide removed)

**DETAILED DESCRIPTION OF THE INVENTION**

[0018] "Antibody": As used herein, the term "antibody" refers to a molecule, preferably a protein, which is capable of specifically binding an antigen, typically and preferably by binding an epitope or antigenic determinant of said antigen, or a hapten. The term antibody also refers to an antigen or hapten binding molecule comprising at least one variable region, wherein said molecule comprises at least one HCVR and/or at least one LCVR. Furthermore, the term antibody refers to an antigen or hapten binding molecule comprising at least one or exactly two antigen binding sites, wherein each of said antigen binding site(s) is formed by one HCVR and one LCVR. Furthermore, the term antibody refers to whole antibodies, such as of the IgG, IgA, IgE, IgM, or IgD class, such as of the IgG class, such as IgG1, IgG2, IgG3, and IgG4, and to antigen binding fragments thereof. Said whole antibodies may comprise either a kappa or a lambda light chain. The term "antibody" also refers to an antigen or a hapten binding antibody fragment, a proteolytic fragment and their recombinant analogue such as a Fab, Fab' and F(ab')2, single domain antibody such as a dAb and VHH (also Nanobody®), Fv, and other mono-, bi- and/or multivalent antibody formats. The term antibody further encompasses a protein comprising at least one or two variable regions, wherein further said protein comprises exactly one HCVR and exactly one LCVR. In a preferred embodiment the term antibody refers to a single chain antibody, preferably to scFv. Thus, preferred antibodies are single chain antibodies, preferably scFvs, disulfide-linked Fvs.
(sdFv) and fragments comprising either a light chain variable region (LCVR) or a heavy chain variable region (HCVR). In the context of the invention the term “antibody” preferably refers to recombinant antibodies, including recombinant proteins consisting of a single polypeptide, wherein said polypeptide comprises at least one, preferably exactly one, variable region. In the context of the invention recombinant antibodies may further comprise functional elements, such as, for example, a linker region, a signal peptide or hydrophobic leader sequence, a detection tag, a moiety binding to a serum protein such as e.g. serum albumin, and/or a purification tag (e.g. myc or His tag).

[0019] “antigen binding site”: An antigen binding site may comprise a HCVR and a LCVR as defined below, wherein said HCVR and said LCVR are located in close proximity and interact in the binding of the antigen. HCVR and LCVR may be located on the same polypeptide molecule, e.g. in case of a scFv. HCVR and the LCVR of an antigen binding site may be located on different polypeptide chains being part of an immunoglobulin, wherein said HCVR is forming part of the heavy chain (referred herein also as “HC”) or of a fragment thereof, and the LCVR is forming part of the light chain (referred herein also as “LC”) or of a fragment thereof.

[0020] “recognizing”: An antibody is said to be “recognizing” an epitope when said antibody is specifically binding an antigen comprising said epitope in a position which is available for interaction with said antibody, and when said antibody does not specifically bind an otherwise identical antigen which does not comprise said epitope, or wherein said epitope is located in a position which is not available for interaction with said antibody. Similarly, an antigen binding site is said to be recognizing an epitope, when an antibody comprising said antigen binding site is recognizing said epitope, wherein typically and preferably said antibody does not comprise a second antigen binding site having a different structure.

[0021] “Fv”: The term Fv refers to the smallest proteolytic fragment of an antibody capable of binding an antigen or hapten and to recombinant analogues of said fragment.

[0022] “single chain antibody”: A single chain antibody is an antibody consisting of a single polypeptide. Preferred single chain antibodies consist of a polypeptide comprising at least one, preferably exactly one VR, wherein preferably said VR is a HCVR. More preferred single chain antibodies consist of a polypeptide comprising a at least one, preferably exactly one, HCVR and at least one, preferably exactly one, LCVR. Still more preferred single chain antibodies comprise exactly one HCVR and exactly one LCVR. Most preferred single chain antibodies are scFv, wherein said scFv consist of a single polypeptide comprising exactly one
HCVR and exactly one LCVR, wherein said HCVR and said LCVR are linked to each other by a linker region, wherein preferably said linker region consists of at least 15, preferably of 15 to 20 amino acids (Bird et al. (1988) Science, 242(4877):423-426). Further preferred single chain antibodies are scFv, wherein said scFv are encoded by a coding region, wherein said coding region, in 5' to 3' direction, comprises in the following order: (1) a light chain variable region (LCVR) consisting of light chain framework (LC FR) 1, complementary determining region (LC CDR) 1, LC FR2, LC CDR 2, LC FR3, LC CDR3 and LC FR4 from a κ or λ light chain; (2) a flexible linker (L), and (3) a heavy chain variable region (HCVR) consisting of framework (HC FR) 1, complementary determining region (HC CDR) 1, HC FR2, HC CDR2, HC FR3, HC CDR3 and HC FR4. Alternatively, single chain antibodies are scFv, wherein said scFv are encoded by a coding region, wherein said coding region, in 5' to 3' direction, comprises in the following order: (1) a heavy chain variable region (HCVR) consisting of framework (HC FR) 1, complementary determining region (HC CDR) 1, HC FR2, HC CDR2, HC FR3, HC CDR3 and HC FR4; (2) a flexible linker (L), and (3) a light chain variable region (LCVR) consisting of light chain framework (LC FR) 1, complementary determining region (LC CDR) 1, LC FR2, LC CDR2, LC FR3, LC DR3 and LC FR4 from a κ or λ light chain.

[0023] “diabody”: The term “diabody” refers to an antibody comprising two polypeptide chains, preferably two identical polypeptide chains, wherein each polypeptide chain comprises a HCVR and a LCVR, wherein said HCVR and said LCVR are linked to each other by a linker region, wherein preferably said linker region comprises at most 10 amino acids (Huston et al. (1988), PNAS 85(16):587958-83; Holliger et al. (1993), PNAS 90(14):6444-6448, Hollinger & Hudson, 2005, Nature Biotechnology 23(9):1126-1136; Arndt et al. (2004) FEBS Letters 578(3):257-261). Preferred linker regions of diabodies comprise 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids.

[0024] “single domain antibody”: The term “single domain antibody” refers to a heavy chain antibody and is used as a general term to include both the full-size antibody, as well as all parts, domains or fragments thereof (including but not limited to antigen-binding domains or fragments such as VHH domains (such as Nanobodies®) or dAbs, respectively). In one embodiment of the invention, the single domain antibodies are light chain variable domain sequences (e.g. a V_L-sequence), or heavy chain variable domain sequences (e.g. a V_H-sequence); more specifically, the single domain antibodies can be heavy chain variable domain sequences that are derived from a conventional four-chain antibody or heavy chain
variable domain sequences that are derived from a heavy chain antibody. According to the invention, the single domain antibodies can be domain antibodies, or immunoglobulin sequences that are suitable for use as domain antibodies, "dAbs", or immunoglobulin sequences that are suitable for use as dAbs, or Nanobodies, including but not limited to VHH sequences, and preferably are Nanobodies. The single domain antibodies provided by the invention are preferably in essentially isolated form (as defined herein), or form part of a protein or polypeptide of the invention (as defined herein), which may comprise or essentially consist of one or more single domain antibodies of the invention and which may optionally further comprise one or more further single domain antibodies (all optionally linked via one or more suitable linkers). For example, and without limitation, the one or more single domain antibodies of the invention may be used as a binding unit in such a protein or polypeptide, which may optionally contain one or more further single domain antibodies that can serve as a binding unit (i.e. against one or more other targets than the antigen of the invention), so as to provide a monovalent, multivalent or multispecific polypeptide of the invention, respectively, all as described herein. Such a protein or polypeptide may also be in essentially isolated form (as defined herein). The invention includes single domain antibodies of different origin, comprising mouse, rat, rabbit, donkey, human and camelid immunoglobulin sequences. The invention also includes fully human, humanized, sequence optimized, affinity matured or chimeric sequences. For example, the invention comprises camelid single domain antibodies and humanized camelid single domain antibodies, or camelized domain antibodies, e.g. camelized dAb as described by Ward et al (Ward ES, Gussow D, Griffiths AD, Jones PT, Winter G (1989) Binding activities of a repertoire of single immunoglobulin variable domains secreted from Escherichia coli. Nature 341:544–546; see also for example WO 94/04678 and Davies and Riechmann (1994 and 1996)). Moreover, the invention comprises fused single domain antibodies, e.g. forming a multivalent and/or multispecific construct (for multivalent and multispecific polypeptides containing one or more V_{HH} domains and their preparation, reference is also made to Conrath et al., J. Biol. Chem., Vol. 276, 10. 7346-7350, 2001, as well as to for example WO 96/34103 and WO 99/23221), and immunoglobulin sequences comprising tags or other functional moieties, e.g. toxins, labels, radiochemicals, etc., which are derivable from the immunoglobulin sequences of the present invention.

[0025] “human antibody”: As used herein, the term “human antibody” refers to an antibody, preferably a recombinant antibody, essentially having the amino acid sequence of a human immunoglobulin, or a fragment thereof, and includes antibodies isolated from human.
immunoglobulin libraries. The term human antibody comprises also an antibody that is not able to bind to any of its receptors as long as the sequence is essentially human.

[0026] **sequence optimized binding agent**: As used herein, the term “sequence optimized binding agent” refers to a binding agent such as e.g. a polypeptide comprising an antibody of the invention as described herein or essentially such as e.g. an antibody of the invention as described herein, essentially having the amino acid sequence of an immunoglobulin, or a fragment thereof, and includes e.g. the binding agent isolated from human immunoglobulin libraries. In the context of the invention “sequence optimized binding agent” may comprise a limited number of amino acid exchanges as compared to the sequence of a native binding agent (native = originally isolated binding agent by the methods of the invention as described herein and/or known to the skilled person in the art). Such amino acid exchanges can, for example, be caused by cloning procedures or may be introduced in order to optimize the stability, binding, functional inhibition, manufacturability, solubility of the native binding agent or because of other reasons. However, the number of such amino acid exchanges in binding agents of the invention is preferably minimized. Preferably, the amino acid sequence of binding agents is at least 85 %, preferably 90 %, more preferably 95 %, even more preferably at least 96 %, still more preferably 97 %, again still more preferably 98 %, again still more preferably 99 % and most preferably 100 % identical to that of the native binding agents. More preferably, the amino acid sequence of the binding agents is at least 85 %, preferably 90 %, more preferably 95 %, even more preferably at least 96 %, still more preferably 97 %, again still more preferably 98 %, again still more preferably 99 % and most preferably 100 % identical to that of native binding agents which are specifically binding to the antigen of interest.

[0027] Preferred sequence optimized binding agents such as e.g. recombinant binding agents differ from native binding agents in at most 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid. Very preferably, differences in the amino acid sequence of binding agents such as e.g. recombinant binding agents and native binding agents are eliminated by means of molecular cloning, and thus, most preferably, the amino acid sequence of a binding agent such as e.g. recombinant binding agent and native binding agent is identical.

[0028] Preferred sequence optimized binding agents comprise (a) least one, preferably one, HCVR, (b) at least one, preferably one, HCCR, (c) at least one, preferably one, LCVR, and (d) at least one, preferably one, LCCR, wherein said at least one HCVR, and/or said at least one HCCR, and/or said at least one LCVR, and/or said at least one LCCR are at least 85%,
preferably 90%, more preferably 95 %, still more preferably at least 96 %, again still more preferably 97 %, again still more preferably 98 %, again still more preferably 99 %, and most preferably 100 % identical to the respective native binding agent regions.

[0029] It is well established that the constant regions of immunoglobulins, including human immunoglobulins, exist in various allotypes, i.e. that the amino acid sequence of said constant regions may differ to a certain extent between individuals of a population. Allotypes of the constant regions of human immunoglobulins are very well studied and the sequence information is readily available to the artisan from various sources, including the Immuno Genetics Information System (http://imgt.cines.fr/).

[0030] "monoclonal antibody": As used herein, the term "monoclonal antibody" refers to an antibody population comprising only one single antibody species, i.e. antibodies having an identical amino acid sequence.

[0031] "constant region (CR)". The term “constant region” refers to a light chain constant region (LCCR) or a heavy chain constant region (HCCR) of an antibody. Typically and preferably, said CR comprises one to four immunoglobulin domains characterized by disulfide stabilized loop structures.

[0032] light chain constant region (LCCR): The LCCR, more specifically the kappa LCCR or the lambda LCCR, typically represents the C-terminal half of a native kappa or lambda light chain of an native antibody. A LCCR typically comprises about 110 amino acids representing one immunoglobulin domain.

[0033] heavy chain constant region (HCCR): The constant region of a heavy chain comprises about three quarters or more of the heavy chain of an antibody and is situated at its C-terminus. Typically the HCCR comprises either three or four immunoglobulin domains. Preferred HCCRs are selected from gamma HCCR, alpha HCCR, epsilon HCCR, my HCCR, and delta HCCR. Very preferred are gamma HCCR, wherein preferably said gamma HCCR is selected from gamma 1 HCCR, gamma 2 HCCR, gamma 3 HCCR, and gamma 4 HCCR, wherein most preferably said gamma HCCR is a gamma 2 HCCR.

[0034] "variable region (VR)". Refers to the variable region or variable domain of an antibody, more specifically to the heavy chain variable region (HCVR) or to the light chain variable region (LCVR). Typically and preferably, a VR comprises a single immunoglobulin domain. Preferred VRs are VRs of immunoglobulins, preferably of human immunoglobulins, wherein further preferably said immunoglobulins, preferably said human immunoglobulins, are selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM, and IgD.
VRs of various species are known in the art. Preferred VRs are human VRs, wherein the framework of said human VRs exhibit at least 80%, preferably at least 85%, more preferably 90%, again more preferably at least 95%, most preferably at least 99% sequence identity with the framework of any known human VR sequence. Preferred VRs are human VRs, wherein the framework of said human VRs exhibit at least 80%, preferably at least 85%, more preferably 90%, again more preferably at least 95%, most preferably at least 99% sequence identity with the framework of any human VR sequence available from public databases, most preferably with any human VR sequence available from the Immunogenetics Information System (http://imgt.cines.fr/).

[0035] Each VR comprises so called complementarity determining regions (CDRs) which are determining the binding characteristics of the antibody and which are embedded in the so called framework. Typically and preferably, VRs comprise three CDRs, preferably CDR1, CDR2, and CDR3, which are embedded into the framework (FR 1-4). Thus, in a preferred embodiment, a VR comprises the following elements in the following order from the N- to the C-terminus: FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4.

[0036] Generally VRs comprise or preferably consist of a polypeptide, wherein said polypeptide is a product of a member of a family of V-gene segments in combination with further gene segments as, for example, D and J gene segments (HCVR) or J gene segments (LCDR).

[0037] "light chain variable region (LCVR)"; Light chain variable regions are encoded by rearranged nucleic acid molecules and are either a kappa LCVR or a lambda LCVR. Human kappa LCVRs comprise a polypeptide, wherein said polypeptide is a product of a member of family 1 to 7 of human kappa V-gene segments. In the context of the invention preferred kappa LCVRs are human kappa LCVRs, preferably human kappa LCVRs which are encoded by a DNA which can be amplified from human B cells using a primer combination of any one of the oligonucleotides disclosed as SEQ ID NO:49 to 52 of WO2008/055795A1 with any one oligonucleotide disclosed as SEQ ID NO:53 to 56 of WO2008/055795A1, and further preferably, PCR conditions described in Example 3 of WO2008/055795A1.

[0038] Human lambda LCVRs comprise a polypeptide, wherein said polypeptide is a product of a member of family 1 to 11 of human lambda V-gene segments. In the context of the invention preferred lambda LCVRs are human lambda LCVRs, preferably human lambda LCVRs which are encoded by a DNA which can be amplified from human B cells using a primer combination of any one of SEQ ID NO:57 to 65 of WO2008/055795A1 with any one

[0039] Typically and preferably, LCVRs comprise three LC CDRs, preferably LC CDR1, LC CDR2, and LC CDR3, which are embedded into the light chain framework (LC FR 1-4). Thus, in a preferred embodiment, a LCVR comprises the following elements in the following order from the N- to the C-terminus: LC FR1 - LC CDR1 -LC FR2 - LC CDR2 - LC FR3 - LC CDR3 - LC FR4.

[0040] "heavy chain variable region (HCVR)": Heavy chain variable regions are encoded by rearranged nucleic acid molecules. Human HCVRs comprise a polypeptide, wherein said polypeptide is a product of a member of family 1 to 7 of human lambda V-gene segments. In the context of the invention preferred HCVRs are human HCVRs, preferably human HCVRs which are encoded by a DNA which can be amplified from human B cells using a primer combination of any one of SEQ ID NO:42 to 47 of WO2008/055795A1 with SEQ ID NO:48 of WO2008/055795A1 and, further preferably, PCR conditions described in Example 3 of WO2008/055795A1.

[0041] Typically and preferably, HCVRs comprise three HC CDRs, preferably HC CDR1, HC CDR2, and HC CDR3, which are embedded into the heavy chain framework (HC FR 1-4). Thus, in a preferred embodiment, a HCVR comprises the following elements in the following order from the N- to the C-terminus: HC FR1 - HC CDR1 -HC FR2 - HC CDR2 - HC FR3 - HC CDR3 - HC FR4.

[0042] "CDR": The complementarity determining region (CDR) 1, 2 and 3 of the HCVR and of the LCVR, respectively, of an antibody can be identified by methods generally known in the art. For the purpose of this application, CDR and FR boundaries are defined as set forth by Scavenger et al. 1999 (Exp Clin Immunogenet., Vol. 16 pp. 234-240), or by Lefranc et al. 2003 (Developmental and Comparative Immunology Vol. 27 pp. 55-77).

[0043] "sequence identity": For the purposes of comparing two or more nucleotide sequences, the percentage of "sequence identity" between a first nucleotide sequence and a second nucleotide sequence may be calculated by dividing [the number of nucleotides in the first nucleotide sequence that are identical to the nucleotides at the corresponding positions in the second nucleotide sequence] by [the total number of nucleotides in the first nucleotide sequence] and multiplying by [100%], in which each deletion, insertion, substitution or addition of a nucleotide in the second nucleotide sequence - compared to the first nucleotide sequence - is considered as a difference at a single nucleotide (position). Alternatively, the
degree of sequence identity between two or more nucleotide sequences may be calculated using a known computer algorithm for sequence alignment such as NCBI Blast v2.0, using standard settings. Some other techniques, computer algorithms and settings for determining the degree of sequence identity are for example described in WO 04/037999, EP 0 967 284, EP 1 085 089, WO 00/55318, WO 00/78972, WO 98/49185 and GB 2 357 768-A. Usually, for the purpose of determining the percentage of "sequence identity" between two nucleotide sequences in accordance with the calculation method outlined hereinabove, the nucleotide sequence with the greatest number of nucleotides will be taken as the "first" nucleotide sequence, and the other nucleotide sequence will be taken as the "second" nucleotide sequence. For the purposes of comparing two or more amino acid sequences, the percentage of "sequence identity" between a first amino acid sequence and a second amino acid sequence (also referred to herein as "amino acid identity") may be calculated by dividing [the number of amino acid residues in the first amino acid sequence that are identical to the amino acid residues at the corresponding positions in the second amino acid sequence] by [the total number of amino acid residues in the first amino acid sequence] and multiplying by [100%], in which each deletion, insertion, substitution or addition of an amino acid residue in the second amino acid sequence - compared to the first amino acid sequence - is considered as a difference at a single amino acid residue (position), i.e. as an "amino acid difference" as defined herein. Alternatively, the degree of sequence identity between two amino acid sequences may be calculated using a known computer algorithm, such as those mentioned above for determining the degree of sequence identity for nucleotide sequences, again using standard settings. Usually, for the purpose of determining the percentage of "sequence identity" between two amino acid sequences in accordance with the calculation method outlined hereinabove, the amino acid sequence with the greatest number of amino acid residues will be taken as the "first" amino acid sequence, and the other amino acid sequence will be taken as the "second" amino acid sequence.

[0044] The terms "cross-block", "cross-blocked" and "cross-blocking" are used interchangeably herein to mean the ability of an amino acid sequence or other binding agents (such as the antibodies of the invention) to interfere with the binding of other amino acid sequences or binding agents of the invention to the Hepatitis B Virus (HBV) preS1 antigen. The extent to which an amino acid sequence or an other binding agent of the invention is able to interfere with the binding of another to a given target, and therefore whether it can be said to cross-block according to the invention can be determined using competition binding assays.
One particularly suitable quantitative cross-blocking assay uses the Friguet-ELISA of example 3 which can measure the extent of interactions. Another suitable quantitative cross-blocking assay uses another ELISA-based approach to measure competition between amino acid sequences or other binding agents in terms of their binding to the target.

**[0045]** "antigen": As used herein, the term “antigen” refers to a molecule which is bound by an antibody. An antigen is recognized by the immune system and/or by a humoral immune response and can have one or more epitopes, preferably B-cell epitopes, or antigenic determinants.

**[0046]** "Hepatitis B Virus (HBV) preS1 antigen": The term “Hepatitis B Virus (HBV) preS1 antigen” or shortly “preS1 antigen” as used herein refers to any polypeptide comprising the epitope comprised by the amino acid sequence X₁X₂PDWX₃X₄X₅ (SEQ ID NO:34), wherein X₁ to X₅ are any amino acid residue, wherein preferably said epitope is located on said polypeptide in a position which allows interaction between said epitope and said antibody. In a preferred embodiment X₁ is selected from N, E, A, R, or S; and/or X₂ is N or S. In a further preferred embodiment X₁X₂ is selected from NN, EN, AN, RN, or SS. In a further preferred embodiment X₃ is selected from F, L, or H; and/or X₄ is N; and/or X₅ is P or T. In a further preferred embodiment X₃X₄X₅ is selected from FNP, LNP, HNP, or FNT. The term preS1 antigen thus includes polypeptides comprising any one of the sequences X₁X₂PDWDFNP (SEQ ID NO:38), NPDWDFNP (SEQ ID NO:39), ANPDWDFNP (SEQ ID NO:40) or TANPDWFNP (SEQ ID NO:32). Further preferably the term preS1 antigen includes polypeptides comprising or consisting of any one of the amino acid sequences of SEQ ID Nos 18 to 31 and SEQ ID NO:37. preS1 antigen as used herein also refers to protein complexes, subviral- or viral particles and components or fractions thereof comprising said polypeptides, preferably on their surface, and further preferably in an orientation which is allowing interaction with an antibody. Thus, the term preS1 antigen also includes HBV and hereby preferably HBV of genotypes A, A (afr), B, C, C (aus), D, E, F, G and H.

**[0047]** “specifically binding”: The specificity of an antibody relates to the antibody’s capability of specifically binding an antigen or hapten. The specificity of this interaction between the antibody and the antigen/hapten (affinity) is characterized by a binding constant or, inversely, by a dissociation constant (Kd). It is to be understood that the apparent affinity of an antibody to an antigen/hapten in a multivalent interaction depends on the structure of the antibody and of the antigen/hapten, and on the actual assay conditions. The apparent affinity of an antibody to an antigen/hapten in a multivalent interaction may be significantly higher
than in a monovalent interaction due to avidity. Thus, affinity is preferably determined under conditions favoring monovalent interactions. Kd can be determined by methods known in the art. Kd of a given combination of antibody and antigen/hapten is preferably determined by ELISA, wherein a constant amount of immobilized antigen/hapten is contacted with a serial dilution of a known concentration of a purified antibody, preferably a monovalent antibody, for example scFv or Fab fragment. Kd is then determined as the concentration of the antibody where half-maximal binding is observed. In a preferred embodiment Kd is determined by equilibrium dialysis. Alternatively, Kd of a monovalent interaction of an antibody and an antigen/hapten is determined by Biacore analysis as the ratio of on rate (kon) and off rate (koff). Lower values of Kd indicate a more specific binding of the antibody to the antigen/hapten than higher values. In the context of the application, an antibody is considered to be “specifically binding an antigen/hapten”, when the dissociation constant (Kd), preferably determined as described above, and further preferably determined in a monovalent interaction, is at most 1 mM (\(\leq 10^{-3}\) M), preferably at most 1 µM (\(\leq 10^{-6}\) M), most preferably at most 1 nM (\(\leq 10^{-9}\) M). Very preferred are antibodies capable of binding an antigen/hapten with a Kd of less than 100 nM ("low nanomolar range"), wherein further preferably Kd is determined in a monovalent interaction. Further preferred antibodies are capable of binding an antigen/hapten with a Kd of 1 to 1000 pM, more preferably of 5 to 800 pM, still more preferably of 5 to 200 pM, most preferably of 5 to 100 pM, wherein further preferably Kd is determined in a monovalent interaction.

[0048] "neutralizing": An antibody of the invention is said to be capable of neutralizing HBV when it is capable of inhibiting, i.e. preventing or slowing down, the replication of HBV upon interaction with said HBV. The neutralizing effect of an antibody of the invention can be observed in vivo and in vitro. Typically and preferably, an antibody is said to be capable of neutralizing HBV when the replication of HBV in said subject is inhibited upon administration of said antibody to said subject, wherein preferably said antibody is administered in an effective amount. The neutralizing effect of an antibody of the invention on HBV may also be assayed in vitro in a cellular system, wherein preferably said assay is performed essentially as set forth in Example 7.

[0049] "effective amount": The effective amount of an antibody of the invention or of a pharmaceutical composition of the invention generally refers to an amount necessary to achieve, at dosages and periods of time necessary, the desired result. In the context of the invention the desired therapeutic result is a therapeutic and/or prophylactic effect on HBV
infection. More specifically the desired effect is the neutralization of HBV in vivo in an HBV infected subject. With respect to the treatment of a human, an “effective amount” typically refers to an amount which does not exceed 1000 mg, preferably said amount is 1 to 500, more preferably said amount is 5 to 100 mg of said antibody.

**[0050]**  **“binding molecule”:** The term binding molecule refers to the antigen or hapten binding antibody of the invention, preferably to fragments and their recombinant analogues such as single chain and/or single domain antibodies, most preferably to scFv comprising the light chain variable region (LCVR) or the heavy chain variable region (HCVR) of the invention. The term binding molecule further encompasses a protein comprising at least one, preferably two variable regions, wherein further preferably said protein comprises exactly one HCVR and exactly one LCVR. In a preferred embodiment the term binding molecule refers to a single domain antibody or single chain antibody, preferably to single chain antibody such as e.g. scFv or scFv-Fc as e.g. described in the experimental part. Thus, preferred binding molecules are single domain antibodies, single chain antibodies, preferably scFvs, disulfide-linked Fvs (sdFv) and fragments comprising either a light chain variable region (LCVR) or a heavy chain variable region (HCVR). In the context of the invention the term “binding molecule” preferably refers to recombinant antibody fragments, including recombinant proteins consisting of a single polypeptide, wherein said polypeptide comprises at least one, preferably exactly one, variable region. In the context of the invention recombinant antibody fragment may further comprise functional elements, such as, for example, a linker region, a signal peptide or hydrophobic leader sequence, a detection tag, a half life extension moiety (Fc fusion, serum albumin fusion, serum albumin binder, pegylation etc), and/or a purification tag (e.g. Fc, myc, his tags) wherein Fc is as defined herein, e.g. is not binding to FcgR. In another aspect the invention also comprises constructs of the above binding molecules such as a construct comprising two identical or non identical units, or constructs comprising one or more binding molecules optionally also comprising additional elements as e.g. described above. In a preferred embodiment the binding molecule is single chain scFv or a single domain antibody with or without Fc part wherein the Fc part if present is as described herein, e.g. does not bind to FcgR.

**[0051]** Generally, by "Fc receptor" is meant any receptor for or recognized by a protein or polypeptide with an immunoglobulin fold or an immunoglobulin domain. In particular, by Fc receptor is meant any receptor for or recognized by an immunoglobulin, and in particular any receptor for or recognized by the immunoglobulins IgG, IgM, IgE and/or IgA. The Fc
receptor may in particular be one of the following receptors: FcRn, Pi gR, Fc alpha R1 (CD89, FcaR1), Fc gamma R1 (CD64, FcgR1), Fc gamma R2 (CD32, FcgR2), Fc gamma R3 (CD16, FcgR3) or Fc epsilon R1 (FceR1). In a preferred aspect of the invention, the FcR binding regions of the antibodies or binding agents of the invention are if present not binding to e.g. the high affinity FcR (i.e. FcgR1). The mutations reducing FcR binding may be e.g. in the CH2 domain in the 2 regions described by Armour et al., 1999 – (see supra). Other interactions to other FcRs such as FcgR2 and FcgR3 may also be affected.

[0052] In one aspect, the invention relates to binding molecule comprising an antibody specifically binding the Hepatitis B Virus (HBV) preS1 antigen, wherein said antibody comprises at least one antigen binding site, wherein said antigen binding site comprises: (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and (b) a LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID NO:12, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14.

[0053] In a further aspect, the invention relates to binding molecule comprising an antibody specifically binding Hepatitis B Virus (HBV) preS1 antigen, wherein said antibody comprises at least one antigen binding site, wherein said antigen binding site comprises: (a) a HCVR, wherein said HCVR comprises a HC CDR1, a HC CDR2 and a HC CDR3; and (b) a LCVR, wherein said LCVR comprises a LC CDR1, a LC CDR2 and a LC CDR3; wherein the CDRs of said at least one antigen binding site, when taken as a whole, differ from the CDRs of an antigen binding site comprising: (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and (b) a LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID NO:12, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14; in at most 3, preferably in at most 2, and most preferably in at most 1 amino acid residue(s).

[0054] In a further preferred aspect, the invention relates to a binding molecule comprising an antibody that comprises at least one antigen binding site, wherein said antigen binding site comprises: (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and (b) a LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID NO:12 or 35, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14 or 36. In a preferred aspect, the invention relates to a binding molecule comprising an antibody that a) is either not able to bind to or only able to bind in a reduced manner to various cell receptors it normally binds such as Fc receptors
(e.g. to FcgR) and/or b) has a flexible hinge region directly adjacent to the antigen binding site, such as in a single chain antibody Fc fusion protein, unlike in an IgG such as e.g. an IgG1, where the hinge region is not directly adjacent to the antigen binding site but separated from it by the CH1 domain, and wherein said binding agent comprises an antibody that comprises at least one antigen binding site, wherein said antigen binding site comprises: (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and (b) a LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID NO:12 or 35, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14 or 36.

[0055] In a further preferred aspect, the invention relates to a binding molecule comprising an antibody that comprises at least one antigen binding site, wherein said antigen binding site comprises: (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and (b) a LCVR, wherein (i) said LCVR comprises the LC CDR1 of SEQ ID NO:12, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14, or (ii) said LCVR comprises the LC CDR1 of SEQ ID NO:35, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:36. In a preferred aspect, the invention relates to a binding molecule comprising an antibody that a) is either not able to bind to or only able to bind in a reduced manner to various cell receptors it normally binds such as Fc receptors (e.g. to FcgR) and/or b) has a flexible hinge region directly adjacent to the antigen binding site, such as in a single chain antibody Fc fusion protein, unlike in an IgG such as e.g. an IgG1, where the hinge region is not directly adjacent to the antigen binding site but separated from it by the CH1 domain, and wherein said binding agent comprises an antibody that comprises at least one antigen binding site, wherein said antigen binding site comprises: (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and (b) a LCVR, wherein (i) said LCVR comprises the LC CDR1 of SEQ ID NO:12, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14, or (ii) said LCVR comprises the LC CDR1 of SEQ ID NO:35, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:36.

[0056] In a further preferred aspect, the invention relates to a binding molecule comprising an antibody that comprises at least one antigen binding site, wherein said antigen binding site comprises: (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and (b) a LCVR, wherein
said LCVR comprises the LC CDR1 of SEQ ID NO:12, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14. In a preferred aspect, the invention relates to a binding molecule comprising an antibody that a) is either not able to bind to or only able to bind in a reduced manner to various cell receptors it normally binds such as Fc receptors (e.g. to FcgR) and/or b) has a flexible hinge region directly adjacent to the antigen binding site, such as in a single chain antibody Fc fusion protein, unlike in an IgG such as e.g. an IgG1, where the hinge region is not directly adjacent to the antigen binding site but separated from it by the CH1 domain, and wherein said binding agent comprises an antibody that comprises at least one antigen binding site, wherein said antigen binding site comprises: (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and (b) a LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID NO:12, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14.

[0057] In a further preferred aspect, the invention relates to a binding molecule comprising an antibody that comprises at least one antigen binding site, wherein said antigen binding site comprises: (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and (b) a LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID NO:35, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:36. In a preferred aspect, the invention relates to a binding molecule comprising an antibody that a) is either not able to bind to or only able to bind in a reduced manner to various cell receptors it normally binds such as Fc receptors (e.g. to FcgR) and/or b) has a flexible hinge region directly adjacent to the antigen binding site, such as in a single chain antibody Fc fusion protein, unlike in an IgG such as e.g. an IgG1, where the hinge region is not directly adjacent to the antigen binding site but separated from it by the CH1 domain, and wherein said binding agent comprises an antibody that comprises at least one antigen binding site, wherein said antigen binding site comprises: (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and (b) a LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID NO:35, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:36.

[0058] In a preferred aspect, the invention relates to a binding molecule comprising an antibody wherein at least one antigen binding site recognizes an epitope comprised by the amino acid sequence $X_1X_2PDWX_3X_4X_5$ (SEQ ID NO:34), wherein $X_1$ to $X_5$ are any amino
acid residue. In a further preferred embodiment $X_1$ is selected from N, E, A, R, or S; and/or $X_2$ is N or S. In a further preferred aspect $X_1X_2$ is selected from NN, EN, AN, RN, or SS. In a further preferred aspect $X_3$ is selected from F, L, or H; and/or $X_4$ is N; and/or $X_5$ is P or T. In a further preferred aspect $X_3X_4X_5$ is selected from FNP, LNP, HNP, or FNT. In a further preferred aspect said at least one antigen binding site recognizes an epitope comprised by the amino acid sequence $X_1X_2PDWDFNP$ (SEQ ID NO:38), wherein $X_1$ and $X_2$ are any amino acid residue. In a further preferred aspect said at least one antigen binding site recognizes an epitope comprised by the amino acid sequence $NPDWDFNP$ (SEQ ID NO:39). In a further preferred aspect said at least one antigen binding site recognizes an epitope comprised by the amino acid sequence $ANPDWDFNP$ (SEQ ID NO:40). In a further preferred aspect said at least one antigen binding site recognizes an epitope comprised by the amino acid sequence $TANPDWFNP$ (SEQ ID NO:32). In a preferred aspect, the invention relates to a binding molecule comprising an antibody that a) is either not able to bind to or only able to bind in a reduced manner to various cell receptors it normally binds such as Fc receptors (e.g. to FcγR) and/or b) has a flexible hinge region directly adjacent to the antigen binding site, such as in a single chain antibody Fc fusion protein, unlike in an IgG such as e.g. an IgG1, where the hinge region is not directly adjacent to the antigen binding site but separated from it by the CH1 domain, and wherein said binding agent comprises an antibody that comprises at least one antigen binding site, wherein said antigen binding site recognizes an epitope comprised by the amino acid sequence $X_1X_2PDWX_3X_4X_5$ (SEQ ID NO:34), wherein $X_1$ to $X_5$ are any amino acid residue. In a further preferred embodiment $X_1$ is selected from N, E, A, R, or S; and/or $X_2$ is N or S. In a further preferred aspect $X_1X_2$ is selected from NN, EN, AN, RN, or SS.

[0059] In a further preferred aspect, the invention relates to a binding molecule comprising an antibody that is cross-blocked by an antibody of the aspects described herein.

[0060] In a further aspect said antibody is a human antibody, preferably a fully human antibody. Thus, in a preferred embodiment said antibody comprises at least one antigen binding site, wherein said antigen binding site comprises the HCVR of SEQ ID NO:7 and the LCVR of any one of SEQ ID NOs 5 or 33. In a further preferred embodiment said antibody comprises at least one antigen binding site, wherein said antigen binding site comprises the HCVR of SEQ ID NO:7 and the LCVR of SEQ ID NO:5.

[0061] In a further aspect said antibody is a monoclonal antibody, wherein typically and preferably, said monoclonal antibody is recombinantly produced. The antibodies of the
invention can be expressed in any naturally occurring or synthetic format, preferably as single domain antibody or scFv. The following thus explicitly refers to all aspects and embodiments of the invention. In a preferred aspect the antibody is in a format selected from the group consisting of: (a) single chain antibody, preferably scFv; (b) Fab fragment; (c) F(ab')2 fragment; (d) scFv-Fc fusion wherein Fc is as defined herein; (e) IgG1; (f) IgG2; (g) IgG3; (h) IgG4; (i) IgA; (j) IgE; (k) IgM; (l) IgD; (m) diabody and (n) single domain antibody, preferably VHH or sequence optimized variants thereof; and wherein optionally said immunoglobulins a) is either not able to bind to or only able to bind in a reduced manner to various cell receptors it normally binds such as Fc receptors (e.g. to FcgR) and/or b) has a flexible hinge region directly adjacent to the antigen binding site, such as in a single chain antibody Fc fusion protein, unlike in an IgG such as e.g. an IgG1, where the hinge region is not directly adjacent to the antigen binding site but separated from it by the CH1 domain.

[0062] In a preferred aspect said antibody is a single chain antibody comprising exactly one HCVR and exactly one LCVR, wherein said HCVR is SEQ ID NO:7, and wherein said LCVR is SEQ ID NO:5 or 33, preferably SEQ ID NO:5. In a further preferred aspect said single chain antibody is produced in the format of an Fc-fusion, preferably of a human scFv-Fcγ1 fusion, wherein said fusion protein a) is either not able to bind to or only able to bind in a reduced manner to various cell receptors it normally binds such as Fc receptors (e.g. to FcgR) and/or b) has a flexible hinge region directly adjacent to the antigen binding site, such as in a single chain antibody Fc fusion protein, unlike in an IgG such as e.g. an IgG1, where the hinge region is not directly adjacent to the antigen binding site but separated from it by the CH1 domain.

[0063] In a very preferred aspect, said single chain antibody comprises or preferably consists of polypeptides selected from the group consisting of amino acid sequences that are 80%, more preferably 85%, more preferably 90%, even more preferably 95%, even more preferably 99%, most preferred 100% identical to SEQ ID NO:3. In a further very preferred aspect said antibody comprises or preferably consists of a peptide, wherein said peptide is encoded by the nucleic acid of any one of SEQ ID NOs 1 or 2.

[0064] In a very preferred aspect, said single chain antibody comprises or preferably consists of polypeptides selected from the group consisting of amino acid sequences that are 80%, more preferably 85%, more preferably 90%, even more preferably 95%, even more preferably 99%, most preferred 100% identical to SEQ ID NO:44.
[0065] In a very preferred aspect, said single chain antibody comprises or preferably consists of polypeptides selected from the group consisting of amino acid sequences that are 80%, more preferably 85%, more preferably 90%, even more preferably 95%, even more preferably 99%, most preferred 100% identical to SEQ ID NO:43.

[0066] In a further aspect said antibody is an IgG, preferably an IgG1, and most preferably a fully human IgG1, wherein said IgG1 optionally is so optimized that a) is either not able to bind to or only able to bind in a reduced manner to various cell receptors it normally binds such as Fc receptors (e.g. to FcgR) and/or b) has a flexible hinge region directly adjacent to the antigen binding site, such as in a single chain antibody Fc fusion protein, unlike in an IgG such as e.g. an IgG1, where the hinge region is not directly adjacent to the antigen binding site but separated from it by the CH1 domain. Thus, in a preferred aspect said antibody comprises two, preferably exactly two, gamma 1 heavy chains, wherein further preferably said gamma 1 heavy chains are identical, and wherein still further preferably at least one, preferably both of said gamma 1 heavy chains comprise or preferably consist of residues 20 to 470 of SEQ ID NO:11 with e.g. mutations as outlined in Figure 6.

[0067] In a further preferred aspect said antibody comprises two, preferably exactly two kappa light chains, wherein further preferably said two kappa light chains are identical, and wherein still further preferably at least one, preferably both of said kappa light chains comprise or preferably consist of residues 21 to 240 of SEQ ID NO:9.

[0068] Thus, in a very preferred aspect said antibody is a fully human IgG1, wherein said IgG1 comprises two gamma 1 heavy chains comprising or consisting of residues 20 to 470 of SEQ ID NO:11 with e.g. mutations as outlined in Figure 6, and two kappa light chains comprising or consisting of residues 21 to 240 of SEQ ID NO:9; and wherein optionally said IgG1 a) is either not able to bind to or only able to bind in a reduced manner to various cell receptors it normally binds such as Fc receptors (e.g. to FcgR) and/or b) has a flexible hinge region directly adjacent to the antigen binding site, such as in a single chain antibody Fc fusion protein, unlike in an IgG such as e.g. an IgG1, where the hinge region is not directly adjacent to the antigen binding site but separated from it by the CH1 domain.

[0069] Due to their specific interaction with the novel preS1 epitope described above, which is conserved over a broad spectrum of HBV genotypes, the antibodies of the invention are capable of specifically binding preS1 antigen or fragments thereof comprising said epitope from a broad spectrum of HBV genotypes. In a preferred embodiment, the antibody is specifically binding preS1(21-47), wherein preferably said preS1(21-47) is selected from any
one of SEQ ID NOs 18 to 25, and wherein further preferably said preS1(21-47) is selected from any one of SEQ ID NOs 19 to 22, and wherein still further preferably said preS1(21-47) is of genotype D (SEQ ID NO:22).

[0070] The dissociation constant of the interaction of an antibody of the invention with an antigen is preferably determined in a monovalent interaction, preferably by Friguet-ELISA, most preferably under conditions as set forth in Example 3, wherein the antibody is used in the format of an scFv-Fc fusion, preferably as a human scFv-Fcγ1 fusion, wherein most preferably said antibody is SEQ ID NO:3, and wherein the antigen is used in the format of a GST-preS1(21-47) fusion product. In a preferred aspect said antibody is specifically binding GST-preS1(21-47), preferably GST-preS1(21-47) of genotype D (SEQ ID NO:37), with a Kd value of less than 1000 pM, preferably of less that 500 pM, more preferably of less than 300 pM, still more preferably of less than 200 pM, and still more preferably of less than 100 pM. In a further preferred embodiment said antibody is specifically binding GST-preS1(21-47) (SEQ ID NO:37) with a Kd value of 1 to 1000 pM, preferably of 1 to 500 pM, more preferably of 10 to 300 pM, still more preferably of 20 to 200 pM, and still more preferably of 20 to 150 pM, still more preferably of 50 to 150 pM, and most preferably of 50 to 100 pM, wherein preferably said antibody is in the format of an scFv-Fc fusion, more preferably of a human scFv-Fcγ1 fusion. In a very preferred embodiment said antibody is specifically binding GST-preS1(21-47) of genotype D (SEQ ID NO:37) with a Kd value of 100 pM, wherein preferably said antibody is in the format of an scFv-Fc fusion, more preferably of a human scFv-Fcγ1 fusion, and wherein most preferably said antibody is SEQ ID NO:3.

[0071] Alternatively, the dissociation constant of the interaction of an antibody and/or binding agent of the invention with an antigen is determined using said antibody or binding agent in the format of an IgG, preferably of an IgG1 as described herein, wherein further preferably the dissociation constant is determined by Friguet-ELISA, most preferably under conditions as set forth in Example 3. In a preferred aspect the antibody of the invention is an IgG, preferably an IgG1, and most preferably an IgG1 as described herein and comprises two gamma 1 heavy chains comprising or consisting of residues 20 to 470 of SEQ ID NO:11, and two kappa light chains comprising or consisting of residues 21 to 240 of SEQ ID NO:9, wherein said antibody, and preferably said IgG1 as described herein, is specifically binding GST-preS1(21-47), preferably GST-preS1(21-47) of genotype D (SEQ ID NO:37), with a Kd value of less than 1000 pM, preferably of less that 500 pM, more preferably of less than 300 pM, still more preferably of less than 200 pM. In a further preferred embodiment the antibody
is an IgG, preferably an IgG1, and most preferably an IgG1 comprising two gamma 1 heavy chains comprising or consisting of residues 20 to 470 of SEQ ID NO:11, and two kappa light chains comprising or consisting of residues 21 to 240 of SEQ ID NO:9, wherein said antibody, and preferably said IgG1, is specifically binding GST-preS1(21-47), preferably GST-preS1(21-47) of genotype D (SEQ ID NO:37), with a Kd value of 1 to 1000 pM, preferably of 1 to 500 pM, more preferably of 10 to 300 pM, still more preferably of 50 to 300 pM, and still more preferably of 100 to 300 pM, still more preferably of 150 to 250 pM, and most preferably of 180 to 200 pM.

[0072] In a further preferred aspect said antibody is capable of binding to HBV subviral particles with an EC50 value of less than 100 ng/ml, preferably less than 50 ng/ml, more preferably of less than 20 ng/ml, wherein preferably said antibody is in the format of an scFv-Fc fusion, more preferably of a human scFv-Fcγ1 fusion, wherein most preferably said antibody is SEQ ID NO:3, and wherein further preferably said subviral particles are derived from HBV genotype D, wherein still further preferably the assay conditions are chosen as disclosed in Example 5.

[0073] In a further preferred aspect said antibody is capable of binding to HBV subviral particles with an EC50 value of 1 to 100 ng/ml, preferably of 1 to 50 ng/ml, more preferably of 1 to 20 ng/ml, still more preferably of 5 to 15 ng/ml and most preferably of 12.2 ng/ml, wherein preferably said antibody is in the format of an scFv-Fc fusion, more preferably of a human scFv-Fcγ1 fusion, wherein most preferably said antibody is SEQ ID NO:3, and wherein further preferably said subviral particles are derived from HBV genotype D, wherein still further preferably the assay conditions are chosen as disclosed in Example 5.

[0074] In a further preferred aspect said antibody is capable of neutralizing HBV, wherein typically and preferably the neutralization capacity of said antibody is assayed under conditions as set forth in Example 7. In a very preferred embodiment, said antibody is capable of neutralizing HBV, wherein said HBV is selected from any one of genotypes A, A (afr), B, C, C (aus), D, E, F, G or H, and wherein preferably said HBV is selected from any one of genotypes A, A (afr), B, C, C (aus), and D, and wherein further preferably said HBV is selected from any one of genotypes A, B, C, and D, and wherein most preferably said HBV is genotype D.

[0075] In a very preferred aspect, said antibody is capable of neutralizing HBV, wherein the EC50 value of the neutralizing activity is below 5 nM, preferably below 3 nM, more preferably below 2 nM, still more preferably below 1 nM, and most preferably below 500 pM,
wherein preferably said HBV is selected from any one of genotypes A, A (afr), B, C, C (aus), D, E, F, G or H, and wherein preferably said HBV is selected from any one of genotypes A, A (afr), B, C, C (aus), and D, and wherein further preferably said HBV is selected from any one of genotypes A, B, C, and D, and wherein most preferably said HBV is genotype D, and wherein still further preferably said antibody is in the format of an scFv-Fc fusion, more preferably of a human scFv-Fcγ1 fusion, and wherein most preferably said antibody is SEQ ID NO:3.

[0076] In a very preferred aspect, said antibody is capable of neutralizing HBV, wherein the EC50 value of said neutralizing activity is 10 pM to 5 nM, preferably 10 pM to 3 nM, more preferably 10 pM to 2 nM, still more preferably 10 pM to 1 nM, still more preferably 200 pM to 1 nM, still more preferably 300 to 600 pM, and most preferably about 500 pM, wherein preferably said HBV is selected from any one of genotypes A, A (afr), B, C, C (aus), D, E, F, G or H, and wherein preferably said HBV is selected from any one of genotypes A, A (afr), B, C, C (aus), and D, and wherein further preferably said HBV is selected from any one of genotypes A, B, C, and D, and wherein most preferably said HBV is genotype D, and wherein still further preferably said antibody is in the format of an scFv-Fc fusion, more preferably of a human scFv-Fcγ1 fusion, and wherein most preferably said antibody is SEQ ID NO:3.

[0077] In a further aspect, the invention relates to a nucleic acid encoding a binding agent and/or an antibody disclosed herein. In a preferred embodiment said nucleic acid comprises or consists of any one of SEQ ID NOs 1, 2, 4, 6, 8, or 10. In a very preferred embodiment said nucleic acid comprises or consists of SEQ ID NO: 4 or 6. In a further preferred embodiment said nucleic acid comprises SEQ ID NOs 4 and 6. In a still further preferred embodiment said nucleic acid comprises or consists of any one of SEQ ID NOs 8 or 10. In a still further preferred embodiment said nucleic acid comprises SEQ ID NOs 8 and 10.

[0078] In a further aspect, the invention relates to an expression vector comprising at least one nucleic acid molecule of the invention. Expression vectors suitable for the expression of the binding agent and/or antibodies of the invention are disclosed, for example, in WO2008/055795A1. In a preferred embodiment said expression vector comprises the nucleic acid sequence of SEQ ID NO:4 and/or of SEQ ID NO:6. In a further preferred embodiment said expression vector comprises the nucleic acid sequence of SEQ ID NO:8 and/or of SEQ ID NO:10.
[0079] In a further aspect, the invention relates to a host cell comprising at least one nucleic acid molecule or at least one expression vector of the invention, wherein preferably said host cell is a bacteria cell or an eukaryotic cell. In a preferred embodiment said host cell is a eukaryotic cell selected from (a) yeast cell, (b) insect cell; and (c) mammalian cell, wherein preferably said mammalian cell is selected from HEK-293T cell, CHO cell, and COS cell. Very preferably, said host cell is a HEK-293T cell.

[0080] It is to be understood that the all aspects of the invention and hereby in particular the pharmaceutical compositions, methods and used disclosed in the following relate to any one of the binding agent and/or antibodies and to any one of the technical features disclosed herein.

[0081] The antibody and/or binding agent of the invention referred to in any one of the aspects of the invention, in particular in the context of the pharmaceutical compositions, methods, and uses disclosed below is preferably a binding agent comprising an antibody that comprises an antigen binding site, wherein said antigen binding site comprises: (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and (b) a LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID NO:12, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14, and wherein said at least one antigen binding site recognizes an epitope comprised by the amino acid sequence X_1X_2PDWDX_3X_4X_5 (SEQ ID NO:34), wherein X_1 to X_5 are any amino acid residue; and wherein optionally said antibody a) is either not able to bind to or only able to bind in a reduced manner to various cell receptors it normally binds such as Fc receptors (e.g. to FcgR) and/or b) has a flexible hinge region directly adjacent to the antigen binding site, such as in a single chain antibody Fc fusion protein, unlike in an IgG such as e.g. an IgG1, where the hinge region is not directly adjacent to the antigen binding site but separated from it by the CH1 domain. More preferably, said antibody comprises an antigen binding site, wherein the HCVR of said antigen binding site is SEQ ID NO:7, and wherein the LCVR of said antigen binding site is SEQ ID NO:5. Still more preferably, said antibody is an IgG, preferably an IgG1 and described herein, and wherein said IgG1 comprises two antigen binding sites, wherein each of said antigen binding sites comprises the HCVR of SEQ ID NO:7 and the LCVR of SEQ ID NO:5. Most preferably, said antibody is an IgG1 as described herein, wherein said IgG1 comprises two gamma 1 heavy chains comprising or preferably consisting of residues 20 to 470 of SEQ ID
NO:11, and two kappa light chains comprising or preferably consisting of residues 21 to 240 of SEQ ID NO:9.

[0082] Furthermore, the HBV referred to in any one of the aspects of the invention, in particular in the context of the methods and uses disclosed below is most preferably an HBV selected from any one of genotypes A, A (afr), B, C, C (aus), D, E, F, G or H, and wherein still further preferably said HBV is selected from any one of genotypes A, A (afr), B, C, C (aus), and D, and wherein still further preferably said HBV is selected from any one of genotypes A, B, C, and D, and wherein most preferably said HBV is genotype D.

[0083] The binding agent and/or antibody of the invention can be incorporated into compositions suitable for administration to a subject, preferably to a human. Thus, in a further aspect, the invention relates to a pharmaceutical composition comprising at least one antibody of the invention, wherein preferably said pharmaceutical composition further comprises a pharmaceutically acceptable carrier, diluent or excipient. Pharmaceutically acceptable carriers, diluents and excipients are disclosed, for example, in Remington, The Science and Practice of Pharmacy, 19th edition, Gennaro (ed.), Mack publishing Co., Easton, PA, 1995.

[0084] In a preferred embodiment said pharmaceutical composition further comprises at least one second antibody, wherein preferably said at least one second antibody is specifically binding an HBV antigen.

[0085] The binding agent, antibody and/or the pharmaceutical composition of the invention are preferably administered to a subject, preferably to a human, using standard administration techniques, preferably selected from oral administration, intravenous administration, intraperitoneal administration, subcutaneous administration, pulmonary administration, transdermal administration, intramuscular administration, intranasal administration, buccal administration, sublingual administration, and suppository administration, preferably intravenous administration.

[0086] The antibodies of the invention may be used in passive immunization, preferably of humans, and further preferably against HBV. The antibodies of the invention are therefore useful in the diagnosis, treatment and/or prophylaxis of HBV infection, preferably in humans.

[0087] In a further aspect, the invention relates to a method of passive immunization, preferably against HBV, said method comprising administering to a subject an effective amount of the binding agent, and/or antibody of the invention or an effective amount of the pharmaceutical composition of the invention.
[0088] In a further aspect, the invention relates to a method of diagnosing, treating and/or preventing HBV infection, said method comprising administering to a subject an effective amount of the binding agent and/or antibody of the invention or an effective amount of the pharmaceutical composition of the invention, wherein preferably said subject is a human, and wherein further preferably said subject is infected by HBV or potentially exposed to HBV.

[0089] In a further aspect, the invention relates to the binding agent, antibody of the invention or to the pharmaceutical composition of the invention, for use in a method of passive immunization, preferably against HBV, preferably in a human, wherein further preferably said antibody is to be administered to said human.

[0090] In a further aspect, the invention relates to a binding agent, antibody of the invention or to the pharmaceutical composition of the invention, for use in a method of treatment and/or prophylaxis of HBV infection, wherein preferably said binding agent, antibody or composition is to be administered to a subject, preferably to a human.

[0091] In a further aspect, the invention relates to the use of the binding agent, antibody or pharmaceutical composition of the invention in the manufacture of a medicament for passive immunization, preferably against HBV.

[0092] In a further aspect, the invention relates to a binding agent, an antibody or pharmaceutical composition of the invention for use in a method of passive immunization, preferably against HBV.

[0093] In a further aspect, the invention relates to the use of a binding agent, an antibody or pharmaceutical composition of the invention in the manufacture of a medicament for the diagnosis, treatment and/or prophylaxis of HBV infection, preferably in a human.

[0094] In a further aspect, the invention relates to a binding agent, an antibody or pharmaceutical composition of the invention for use in a method of diagnosing, treating and/or preventing HBV infection, preferably in a human.
EXAMPLE 1

Characterization of the preS1-specific antibodies

[0095] HBV preS1 specific scFv-Fc clones were identified and expressed in 293T cells. To check for preS1-specific binding, ELISA plates were coated with preS1 (21-47)-conjugated RNase A at a concentration of 10 µg/ml in coating buffer (0.1 M NaHCO3, pH 9.6), over night at 4 °C. In parallel, scFv-Fc expression levels were measured by sandwich ELISA. For this, an identical set of plates was coated with Fcγ-specific, goat anti-human F(ab')2 antibody (Jackson ImmunoResearch Laboratories 109-006-098) at a concentration of 2.5 µg/ml. The plates were then washed with wash buffer (PBS / 0.05 % Tween) and blocked for 2 h at 37 °C with 3 % BSA in wash buffer. The plates were then washed again and incubated with 3-fold serial dilutions of the cell culture supernatants, starting at a dilution of 1/10. To permit determination of antibody concentrations in the supernatants, the sandwich ELISA was also carried out with a serial dilution of a control scFv-Fc antibody with known concentration. All dilutions were done in 1 % BSA in wash buffer. Plates were incubated at room temperature for 2 h and then extensively washed with wash buffer. Bound scFv-Fc fusion proteins were then detected by a 1 h incubation with a HRPO-labeled, Fcγ-specific, goat anti-human IgG antibody (Jackson ImmunoResearch Laboratories 109-035-098). After extensive washing with wash buffer, plates were developed with OPD solution (1 OPD tablet, 25 ml OPD buffer and 8 µl 30 % H2O2) for 5 to 10 min and the reaction was stopped with 5 % H2SO4 solution. Plates were then read at OD 450 nm on an ELISA reader (Biorad Benchmark). Antibody 2D028 showed a strong reactivity with preS1 (21-47), with an EC50 of 0.4 ng/ml (approximately 3.7 pM) under these assay conditions. Therefore, the scFv-D028-Fc (SEQ ID NO: 3) expression construct was subjected to sequencing (see also Figure 6 for an overview of the different functional parts of the compound, note that the Fc part is with mutations wherein the mutations reduce the FcR binding and are in the CH2 domain in the 2 regions described in Figure 6 and also described by Armour et al., 1999 – (see supra)). The nucleic acid sequences encoding the light and heavy chain variable regions of an antibody denoted scFv-D028-Fc are depicted in SEQ ID NOs 4 and 6, respectively. The amino acid sequence of the light chain variable region of scFv-D028-Fc is depicted in SEQ ID NO:5 and the amino acid sequence of the heavy chain variable region of scFv-D028-Fc is depicted in SEQ ID NO:7.

[0096] Besides scFv-D028-Fc, seven further independent clones were sequenced, all of which exhibited very high sequence identity to scFv-D028-Fc. For example, C037 comprised
the HCVR of scFv-D028-Fc (SEQ ID NO:7), and the LCVR of SEQ ID NO:33. SEQ ID NO:33 differs from the LCVR of C037 (SEQ ID NO:5) in 3 amino acid residues, one of which is located in LC CDR1 and one of which is located in LC CDR3.

EXAMPLE 2

Expression and purification of the preS1-specific antibody

Expression of the scFv-Fc antibody was done in HEK-293T cells. One day before transfection, 5x10⁶ 293T cells were plated onto a 10 cm tissue culture plate. Cells were then transfected with the scFv-2D028-Fc expression vector using Lipofectamin Plus (Invitrogen) according to the manufacturer’s recommendations, incubated one day, and replated on a 14 cm dish in the presence of 1 μg/ml puromycin. After 3 days of selection, puromycin-resistant cells were transferred to two Poly-L-Lysine coated 14 cm plates. One day later, medium was replaced by serum-free medium and supernatant containing scFv-2D028-Fc protein was collected every 3 days and filtered through a 0.22 μM Millex GV sterile filter (Millipore).

The consecutive harvests were pooled and applied to a protein A-sepharose column. The column was washed with 10 column volumes of phosphate-buffered saline (PBS), and bound protein eluted with 0.1 M Glycine pH 3.6. 1 ml fractions were collected in tubes containing 0.1 ml of 1 M Tris pH 7.5 for neutralization. Protein-containing fractions were analyzed by SDS-PAGE and pooled. The buffer was exchanged with PBS by dialysis using 10’000 MWCO Slide-A-Lyzer dialysis cassettes (Pierce). The purified proteins in PBS were then filtered through 0.22 μM Millex GV sterile filters (Millipore) and aliquoted. Working stocks were kept at 4°C, whereas aliquots for long-term storage were flash-frozen in liquid nitrogen and kept -80°C.

EXAMPLE 3

Determination of affinity by Friguet-ELISA

The dissociation constant (Kd) of antibody binding to preS1 (21-47) of genotype D in solution was determined using an ELISA-based method essentially as described (Friguet B. et al., 1985, J. Immunol. Meth. 77, 305-319). Briefly, a 10 ng/ml solution of scFv-2D028-Fc (SEQ ID NO:3) was incubated in the presence of different concentrations of GST-preS1 (SEQ ID NO:37) (3-fold serial dilutions ranging from 100 nM to 1.7 pM) in PBS / 1 % BSA. After 2 h at room temperature, free antibody was detected by a classical ELISA similar to the one
described in Example 1. For this, ELISA plates that had been coated with RNAse-preS1 conjugate at a concentration of 5 ng/ml at 4°C overnight were washed with wash buffer (PBS / 0.05 % Tween) and blocked for 1 h at 37 °C with 3 % BSA in wash buffer. The plates were then washed again and incubated with the solution binding reactions for 30 min at room temperature. After extensive washing with wash buffer, bound scFv-Fc fusion proteins were detected by a 1 h incubation at room temperature with a HRPO-labeled, Fcγ-specific, goat anti-human IgG antibody (Jackson ImmunoResearch Laboratories 109-035-098). After extensive washing with wash buffer, plates were developed with OPD solution (1 OPD tablet, 25 ml OPD buffer and 8 μl 30 % H₂O₂) for 15 min and the reaction was stopped with 5 % H₂SO₄ solution. Plates were then read at OD 450 nm on an ELISA reader (Biorad Benchmark). The Kd values were determined as the EC50 of the ELISA signal as a function of the preS1 peptide concentration present in the solution binding reaction. The antibody scFv-2D028-Fc (SEQ ID NO:3) was found to have a high affinity for monovalent GST-preS1 (21-47) (SEQ ID NO:37), with a Kd value of about 100 pM (122 pM and 76 pM in two independent experiments).

EXAMPLE 4

Binding to preS1 sequences derived from different HBV genotypes

Cross-reactivity with preS1 sequences derived from different genotypes of HBV was analyzed by testing the binding of scFv-2D028-Fc to solid phase bound peptide variants (analysis performed by Pepscan Presto BV, Lelystad, The Netherlands). Binding to a total of 8 different 27-mer peptides (Table 1), corresponding to the preS1 consensus sequence (SEQ ID NO:18) and 7 peptides covering the 10 major genotypes (SEQ ID NOs 19-25), was assayed at various antibody concentrations. Antibody 2D028 displayed a broad cross-reactivity and bound each of the peptides in a dose-dependent manner (Figure 1). Significantly, the peptides corresponding to the 4 major HBV genotypes A-D were recognized equally well.

Table 1. Variants of the Hepatitis B virus preS1 sequence, amino acid positions 21 to 47. The minimal epitope recognized by the antibodies of the invention is underlined.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sequence (21-47)</th>
<th>SEQ ID NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consensus</td>
<td>PLGFFPDHQLDPAFRANSNNPDEDFNP</td>
<td>18</td>
</tr>
<tr>
<td>A, C, C (aus)</td>
<td>PLGFFPDHQLDPAFGANSNNPDEDFNP</td>
<td>19</td>
</tr>
</tbody>
</table>
EXAMPLE 5

Binding to HBV subviral particles

[00101] There is ample evidence demonstrating binding of antibody scFv-D028-Fc to the preS1 (21-47) peptide, be it coupled to Qβ or RNase A (Example 1), conjugated to a solid phase support (Example 4), or fused to GST (Example 3). However, binding to large hepatitis B surface protein (LHBs), i.e. native surface protein comprising the preS1 and preS2 regions, remained to be shown. Thus, to further characterize the binding properties of antibody 2D028, binding to HBV genotype D subviral particles was investigated by ELISA.

[00102] Subviral particles rich in LHBs were isolated from plasma of HBV-infected individuals as described (Glebe, D. et al., 2003, J. Virol. 77, 9511-9521; Glebe, D. et al., 2005, Gastroenterology 129, 234-245). Wells of an ELISA plate were then coated with the subviral particles at a concentration of 5 μg/ml in coating buffer (0.1 M NaHCO3, pH 9.6), over night at 4°C. After washing with wash buffer (PBS / 0.05 % Tween) the wells were blocked for 90 min at 37 °C with 3 % BSA in wash buffer. The wells were then washed again and incubated with 3-fold serial dilutions of scFv-2D028-Fc in 1 % BSA in wash buffer, starting at a concentration of 250 ng/ml. Antibody was allowed to bind for 2.5 h at room temperature before the wells were extensively washed with wash buffer. Bound antibody was detected by a 45 min incubation with a HRPO-labeled, Fcγ-specific, goat anti-human IgG antibody (Jackson ImmunoResearch Laboratories 109-035-098). After extensive washing with wash buffer, wells were developed with OPD solution (1 OPD tablet, 25 ml OPD buffer and 8 μl 30 % H2O2) for 10 min after which the reaction was stopped with 5 % H2SO4 solution and the absorbance was read at 450 nm on an ELISA reader (Biorad Benchmark). Antibody 2D028 showed a strong reactivity with subviral particles with an EC50 of 12.2 ng/ml (112 pM), demonstrating that it was capable of recognizing preS1 in the context of native LHBs.
Epitope mapping

[00103] The region of preS1 (21-47) recognized was narrowed down by testing the binding of scFv-2D028-Fc to various GST fusion proteins in ELISA. To this end, GST fusion proteins were prepared that contain at their C-terminus either the whole 27-mer preS1 (21-47) peptide, or one of five overlapping 9- to 10-mer peptides (Figure 2A). The amino acid sequences of the GST fusion proteins are disclosed as SEQ ID NOs 26 to 31. Thus, rows of wells of a 96 well ELISA plate were coated with the respective GST fusion protein, at a concentration of 5 μg/ml in coating buffer (0.1 M NaHCO₃, pH 9.6), over night at 4 °C. As negative controls, one row of wells was coated with 5 μg/ml of GST, whereas one was left uncoated. The plate was then washed with wash buffer (PBS / 0.05 % Tween) and blocked for 1 h at 37 °C with 3 % BSA in wash buffer. The plate was then washed again and incubated with serially diluted scFv-2D028-Fc for 90 minutes at 37 °C. After extensive washing with wash buffer, bound scFv-Fc fusion protein was detected by a 45 minute incubation with a HRPO-labeled, Fcγ-specific, goat anti-human IgG antibody (Jackson ImmunoResearch Laboratories 109-035-098). After extensive washing with wash buffer, plates were developed with OPD solution (1 OPD tablet, 25 μl OPD buffer and 8 μl H₂O₂) for 5 minutes and the reaction was stopped with 5 % H₂SO₄ solution. Plates were then read at OD 450 nm on an ELISA reader (Biorad Benchmark). Antibody 2D028 showed strong reactivity with GST-preS1 (21-47) and GST-preS1 (38-47), whereas no significant binding to GST alone or GST fused to any of the other peptides was detected (Figure 2B). This demonstrates that the entire epitope recognized is comprised in the sequence TANPDWDFNP (SEQ ID NO:32), the most C-terminal 10 amino acids of preS1 (21-47). The epitope recognized was further characterized by testing the binding of scFv-2D028-Fc to solid phase bound variants of the peptide TANPDWDFNP (analysis performed by Pepsan Presto BV, Lelystad, the Netherlands). Two types of analysis were done: first, epitope scanning by synthesizing all 36 different 3 to 10 mer variants; and second, point mutations by synthesizing all 190 possible single positional variants. Epitope scanning revealed that the minimal epitope covers most of the 10 amino acids and that the peptide could not be made much shorter without losing significant binding activity (Figure 3A). Only up to two amino acids could be removed from the N-terminus, whereas no C-terminal deletions were possible without completely abolishing binding. Thus, the epitope recognized by 2D028 is comprised in the 8-mer sequence NPDWDFNP. In line with these results, analysis of the single positional variants revealed that the amino acid residues near the C-terminus of the peptide had a stronger influence on binding than those near the N-terminus.
(Figure 3B). Significantly, the central 4 amino acids were crucial for binding and any alterations in this region abolished binding completely. Thus, it appears that the motif PDWD is the core of the epitope, with residues C-terminal to it contributing to binding.

EXAMPLE 7

Neutralizing activity of the preS1-specific scFv-D028-Fc

[00104] HBV genotype D virus particles were isolated from plasma of HBV-infected individuals and quantified as described (Glebe, D. et al., 2003, J. Virol. 77, 9511-9521; Glebe, D. et al., 2005, Gastroenterology 129, 234-245). Primary Tupaia belangeri hepatocytes (PTH) were isolated as described (Glebe, D. et al., 2003, J. Virol. 77, 9511-9521; Glebe, D. et al., 2005, Gastroenterology 129, 234-245) and cultured in Hepatocyte Growth Medium (HGM), consisting of DMEM (Invitrogen, Cat. No. 21063) containing insulin (5 μg/ml), transferrin (5 μg/ml), selenium (5 ng/ml), BSA (2 g/l), glucose (2 g/l), galactose (2 g/l), ornithine (0.1 g/l), proline (30 mg/l), nicotine amide (0.61 g/l), ZnCl₂ (0.544 μg/l), ZnSO₄ × 7 H₂O (0.75 μg/l), CuSO₄ × 5 H₂O (0.2 μg/l), MnSO₄ (0.025 μg/l), 50 μM Glutamax I (Invitrogen), 0.01 μM dexamethasone (Invitrogen), Gentamicin (0.1 g/l), and Amphotericin B (250 μg/l). To determine its neutralizing activity, different amounts of scFv-2D028-Fc were combined with 4x10⁶ genome equivalents (GE) of HBV genotype D in 50 μl HGM in siliconized test tubes, and pre-incubated for 1 hour at 16°C on a shaker. The antibody-HBV mixture was then added to wells of 12 well plates containing PTH (freshly isolated 3 days before) in 250 μl HGM. After one day at 37°C, the supernatants were removed, frozen, and replaced by 600 μl HGM. On days 4, 7, 11, and 15, the supernatants were also removed, frozen, and replaced by 600 μl HGM. Infection rates were determined by measuring the HBsAg concentrations in the supernatants by ELISA as follows. Wells of a 96 well microtiter plate were coated overnight at 4°C with 1 μg/ml of the mouse monoclonal anti-HBs antibody C20/02 in NaPP coating buffer (8.6 mM Na₂HPO₄ x H₂O, 2.2 mM KH₂PO₄, 183 mM NaCl, pH 7.0). The wells were then washed twice with PBS / 0.1 % Tween and twice with PBS, and blocked overnight at 4°C with 10 % fetal bovine serum in TNE (10 mM Tris, 200 mM NaCl, 1 mM EDTA, pH 7.4). The wells were then washed again as above, and incubated with the cell culture supernatants (100 μl/well) overnight at 4 °C. To allow for quantification of HBsAg, one row of an ELISA plate was incubated with a 2-fold serial dilution of purified HBsAg in PBS / 1 % casein (8 dilutions, starting at 10 ng/ml). After an additional wash step as above, bound HBsAg was detected by adding 100 μl per well of a 1 : 40 dilution of anti-HBs-biotin
conjugate I (Kit Enzygnost HBsAg 5.0, Dade-Behring AG, Marburg) in PBS / 0.1 % casein and incubating for 1 h at 37 °C. After washing again, 100 μl of a 1 : 500 dilution of streptavidin-peroxidase conjugate (Dianova) in PBS / 0.1% casein was added to each well, followed by a 30 min incubation at 37 °C. After a final extensive wash, bound antibodies were detected by adding 100 μl TBM X-tra substrate (Kem-En-Tec Diagnostics) per well. After 5 to 15 min, the reactions were stopped by adding 50 μl 0.5 M H₂SO₄ to each well and the plates were read at 450 nm on a microplate photometer (LP 400, Diagnostic Pasteur). Cell viability was analyzed on day 15 by using the WST-1 reagent (Roche) and used to normalize the values obtained.

[00105] A control antibody specific for the virus-like particle Qβ, scFv-Qb3-Fc (Beerli, R. R. et al., 2008, Proc. Natl. Acad. Sci. USA 105, 14336-14341), had no effect on HBV replication even at a concentration of 10 μg/ml, as shown by the presence of substantial amounts of HBsAg in the supernatants on day 15. In striking contrast, scFv-2D028-Fc potently inhibited HBV replication at all concentrations tested (Figure 4A). Substantial inhibition was obvious already at the lowest antibody concentration (0.1 μg/ml), and infection was essentially undetectable at 10 μg/ml of the antibody. Next, antibody 2D028 was titrated in order to determine the 50 % effective concentration (EC50) of inhibition. To this end, HBV was pre-incubated with various scFv-2D028-Fc concentrations, used to infect PTH, and supernatants were collected on days 4, 7, 11, and 15 as described above. Infection rates were determined by measuring the HBsAg concentrations as described above. In some experiments, infection was also monitored by measuring HBeAg concentrations. For this, supernatants harvested on day 11 were analyzed by microparticle enzyme immunoassay (MEIA) on an AxSYM System (Abbott), using the program AxSYM HBe 2.0 and the HBe 2.0 Reagent Pack (Abbott, Cat. No. 7D5220). Cell viability was determined on days 4, 7, 11, and 15 by measuring the urea concentration in the supernatants and used to normalize the values obtained. Antibody 2D028 showed potent neutralizing activity, with an EC50 that was consistently below 100 ng/ml, irrespective of the method used to quantify HBV (Table 2). The EC50 based on HBsAg measurement was at 48 ng/ml (440 pM; average of 4 experiments), whereas the EC50 based on HBeAg measurement was at 52 ng/ml (476 pM; average of 2 experiments).

Table 2. Neutralizing activity of antibody 2D028.
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EXAMPLE 8

Construction, expression, and purification of fully human preS1-specific IgG1

[00106] An expression vector allowing for expression of antibody 2D028 as fully human IgG1κ was generated. Thus, DNA sequences encoding a human γ1 heavy chain and a human κ light chain were produced by total gene synthesis (SEQ ID NOs 8 and 10, by GeneArt AG, Germany). The light chain coding sequence was flanked by a Nhe1 recognition site upstream and Pme1 recognition site downstream of the open reading frame (cf. SEQ ID NO:8). The heavy chain coding sequence was flanked by a Ascl recognition site upstream and a Pac1 recognition site downstream of the open reading frame (cf. SEQ ID NO:10). The amino acid sequence of the entire human κ light chain, including the signal peptide, is depicted in SEQ ID NO:9. The amino acid sequence of the entire human γ1 heavy chain, including the signal peptide, is depicted in SEQ ID NO:11. Light and heavy chain coding regions were then combined into the EBNA-based expression vector pCB15 (disclosed as SEQ ID NO:104 of WO2008/055795A1). Thus, the κ light chain coding region was digested with the restriction enzymes Nhe1 and Pme1, and ligated into Nhe1-Pme1 digested pCB15, generating the plasmid pCB15-LC-κ-2D028. This plasmid was then digested with the restriction enzymes Ascl and Pac1 and ligated to the Ascl-Pac1 digested γ1 heavy chain coding region, generating the plasmid pCB15-IgG1κ-2D028. Expression of IgG1κ-2D028 in HEK-293T cells, as well as purification by protein A-agarose chromatography, was done as described for the scFv-Fc fusion protein (Example 2).

[00107] The dissociation constant (Kd) of the interaction of IgG1κ-2D028 with preS1 (21-47) of genotype D (SEQ ID NO:37) in solution was determined using Friguet ELISA essentially as described in Example 3 above. Kd of IgG1κ-2D028 and preS1 (21-47) of genotype D (SEQ ID NO:37) was found to be about 190 pM (197 pM and 182 pM in two independent experiments).
EXAMPLE 9

The preS1-specific human IgG1-2D028 lacks neutralizing activity

The neutralizing activity of antibody clone 2D028 as a fully human IgG1 was investigated essentially as described in Example 7. As expected, the control scFv-Fc antibody Qb3 had no effect on HBV replication leading to large amounts of HbsAg in the culture supernatants by day 15. Surprisingly, unlike the scFv-Fc antibody, 2D028 had no measurable effect on HBV replication at any of the concentrations tested when applied as a human IgG1 (Figure 4B). Specifically, while the scFv-Fc led to an almost complete inhibition of HBV replication at 1μg/ml (Figure 4A), the IgG1 used at the same concentration had no effect (Figure 4B). The experiment was repeated once, with the same outcome. These results are highly unexpected considering the observation that the in vitro binding properties of 2D028 do not depend on the antibody format (Examples 3 and 8).

EXAMPLE 10

Simultaneous binding of 2D028 and mAb MA18/7 to preS1(21-47)

The mouse mAb MA18/7 has been described previously and binds the epitope DPAF located at the position preS(31-34) (Sominskaya I. et al. (1992) Med. Microbiol. Immunol. 181, 215-226). To test whether the antibody 2D028 is able to bind to preS1(21-47) simultaneously with MA18/7, a sandwich ELISA was carried out (Figure 5). To this end, wells of a 96 well ELISA plate were coated with mAb MA18/7 at a concentration of 2 μg/ml in coating buffer, overnight at 4°C (4 rows of 12 wells). The wells were then washed twice with wash buffer (PBS / 0.05 % Tween) and blocked for 2 h at 37°C with 3 % BSA in wash buffer. After two additional wash steps, the wells were incubated with 0.5 μg/ml of a peptide comprising preS1(21-47) (PLGFFPDHQLDPAFRANTANPDWDFNPNGC; SEQ ID NO:41) in wash buffer/1% BSA for 2h at room temperature. After extensive washing, wells were incubated with serially diluted scFv-Fc antibodies in wash buffer/1% BSA for 1h at room temperature. After extensive washing with wash buffer, bound scFv-Fc antibodies were detected by a 1h incubation with a HRPO-labeled, Fcy-specific, goat anti-human IgG antibody (Jackson ImmunoResearch Laboratories 109-035-098). ELISA wells were, after extensive washing with wash buffer, developed with OPD solution (1 OPD tablet, 25 ml OPD buffer and 8 μl H2O2) for 5 minutes and the reaction was stopped with 5 % H2SO4 solution. Plates were read at OD 450 nm on an ELISA reader (Biorad Benchmark).
It was found that scFv-Fc antibody 2G002 binding the sequence LDPAFRANT (SEQ ID NO:42) comprising the epitope recognized by MA18/7, and scFv-Fc antibody E003 binding an irrelevant control antigen, did not display significant reactivity with preS1(21-47) peptide bound to immobilized MA18/7. In contrast, scFv-Fc antibody 2D028, i.e. scFv-D028-Fc, displayed strong reactivity with MA18/7-preS1(21-47) complexes. This indicates that the peptide preS1(21-47) accommodates simultaneous binding of both antibodies and that the epitopes recognized are functionally independent.

Table 3. Sequences

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PLGFPPDHQLDPARANTANPDWFENPGGC

SEQ ID NO: 42

LDPAFRANT

SEQ ID NO: 43

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SGVPDRFSGSQGSSGTDFLTISSLQAEVAVYCCQQYYNTPSFGQGTKEIKGSSRSSSS
GGGGGSGGGREVLVESGDLVKGSLRLSCAASGLTFSNAWMNWQRQAPGKGLEWVGRIK
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SEQ ID NO: 44

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GGGGGSGGGREVLVESGDLVKGSLRLSCAASGLTFSNAWMNWQRQAPGKGLEWVGRIK
SKSDGGTTDYAAPVEGRFSISRDDSĐTLYLQMNSLKTEDTAVYCASRLVAEGGFDSWQQ
GTIVTVSSASTKGPSVTSQAGRKLTHTCPPCPAEAGAPPVLFPFPKPDITMSRTPE
VTCDVVDHVHDPVFKFNYWYVGDGEVHNAKTKRPQLPQNTYRESVSTVLSQHLDWNLGKEY
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WESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVHEALHNHYTQKSL
SLSPGK
CLAIMS

1. An antibody specifically binding Hepatitis B Virus (HBV) preS1 antigen, wherein said antibody comprises at least one antigen binding site, wherein said antigen binding site comprises:
   (a) a HCVR, wherein said HCVR comprises a HC CDR1, a HC CDR2 and a HC CDR3; and
   (b) a LCVR, wherein said LCVR comprises a LC CDR1, a LC CDR2 and a LC CDR3;
   wherein the CDRs of said at least one antigen binding site, when taken as a whole, differ from the CDRs of an antigen binding site comprising:
      (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and
      (b) a LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID NO:12, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14;
   in at most 3, preferably in at most 2, and most preferably in at most 1 amino acid residue(s).

2. The antibody of claim 1, wherein the antibody is a scFv selected from the group of scFv consisting of amino acid sequences that are 80% identical to SEQ ID NO: 43.

3. The antibody of any one of claims 1 or 2, wherein said antibody is isolated.

4. The antibody of any one of claims 1 to 3, wherein said antibody is a human antibody.

5. The antibody of any one of claims 1 to 4, wherein said antibody comprises at least one antigen binding site, wherein said antigen binding site comprises:
   (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and
   (b) a LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID NO:12, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:14.
6. The antibody of any one of claims 1 to 4, wherein said antibody comprises at least one antigen binding site, wherein said antigen binding site comprises:
   (a) a HCVR, wherein said HCVR comprises the HC CDR1 of SEQ ID NO:15, the HC CDR2 of SEQ ID NO:16, and the HC CDR3 of SEQ ID NO:17; and
   (b) a LCVR, wherein said LCVR comprises the LC CDR1 of SEQ ID NO:35, the LC CDR2 of SEQ ID NO:13, and the LC CDR3 of SEQ ID NO:36.

7. The antibody of any one of claims 1 to 6, wherein said HCVR is SEQ ID NO:7.

8. The antibody of any one of claims 1 to 7, wherein said LCVR is SEQ ID NO:5 or SEQ ID NO:33, and wherein preferably said LCVR is SEQ ID NO:5.

9. The antibody of any one of claims 1 to 6, wherein the antibody is selected from the group of antibodies that have sequence identity of 80%, more preferably 85%, more preferably 90%, even more preferably 95%, even more preferably 99%, most preferred are identical to SEQ ID NO: 3.

10. The antibody of any one of claims 1 to 9, wherein said antibody is specifically binding GST-preS1(21-47) of genotype D (SEQ ID NO:37) with a Kd value of less than 1000 pM, preferably of less that 500 pM, more preferably of less than 300 pM, still more preferably of less than 200 pM.

11. The antibody of any one of claims 1 to 10, wherein said antibody is capable of neutralizing HBV, wherein preferably the EC50 value of the neutralizing activity is below 5 nM, more preferably below 3 nM, still more preferably below 2 nM, still more preferably below 1 nM, and most preferably below 500 pM, wherein further preferably said HBV is selected from any one of genotypes A, A (afr), B, C, C (aus), D, E, F, G or H, and wherein most preferably said HBV is genotype D.

12. A binding agent comprising at least an antibody of any one of claims 1 to 11.
13. A pharmaceutical composition comprising at least one antibody of any one of claims 1 to 11 or a binding agent of claim 12, wherein preferably said pharmaceutical composition further comprises a pharmaceutically acceptable carrier, diluent or excipient.

14. A method of diagnosing, treating and/or preventing HBV infection, said method comprising administering to a subject an effective amount of the antibody of any one of claims 1 to 11, or a binding agent of claim 12, or of the pharmaceutical composition of claim 13, wherein preferably said subject is a human, and wherein further preferably said HBV is selected from any one of genotypes A, A (afr), B, C, C (aus), D, E, F, G or H.

15. The antibody of any one of claims 1 to 11, or a binding agent of claim 12, or the pharmaceutical composition of claim 13 for use in a method of diagnosing, treating and/or preventing HBV infection, preferably in a human, and wherein further preferably said HBV is selected from any one of genotypes A, A (afr), B, C, C (aus), D, E, F, G or H.
Figure 1

HBV Genotype
Figure 2

A

GST-preS1:

preS1 (21-47):
P L G F F P D H Q L D P A F R A N T A N P D W D F N P

Peptides:
P L G F F P D H Q L
P D H Q L D P A F
L D P A F R A N T
F R A N T A N P D
T A N P D W D F N P

B

Graph showing concentration vs. OD450nm for different concentrations of control, GST, and various peptide variants.
Figure 3

A

B
Figure 5

[Graph showing OD-450nm against concentration (ng/ml) with different markers representing 2D028, 2G002, and E003]
Figure 6

vector-derived
DADPAQAAEHOMTQSPDLSLAVSLGERATINCKSSQSVLYSSNNRNLYLAWYQKPP
GQPPKLLYYWAISTRDSGVPRFSGSSTDTFTLTISSLQAEQDVAVVYCCQYNT
PYSFGQQGTKLEIKGSSRSSSSSGGGGGGGGREVQLVESGGDLVKGPSGSLRLSCA
ASGLTFSNAWMNWVRQAPGKGEWVGRIKSKSDGTTDYAAPVEGRFSISRDDS
part of CH1
KDTLYLQMNLSLKDTEAVYCASCRLVAEGBFDSWQGTLYTVSSASTKGPSVT
part of hinge
GQAGRKLTHTCPPCPAPEAEAPSPFVLPNPCPKQDKTMISRTPEVTCVVVDVSHE

part of hinge
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part of hinge
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DPEVKFNWYVDGVEVHNAKTTRPEEQYNSTYRVSVLTVLHQDWLNGKEYKCKKV

SNKALPASTEKTISKAKGQPREQVTLYPPSRDELTKNQVSLTCLVKGFYPDSI

AVEWESNGQPPENNYKTTTTPVLSDGSFFLYSKLTVDKSRGWQGNVFSCSCVMHEA

LHNHTQKSLSLSPGK

mutated residues (with WT above)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K14/02  A61K39/395  A61P31/20

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K  A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>NEURATH A R ET AL: &quot;Antibodies to synthetic peptides from the pre-S1 and pre-S2 regions of one subtype of the hepatitis B virus (HBV) envelope protein recognize all HBV subtypes&quot;, MOLECULAR IMMUNOLOGY, PERGAMON, GB, vol. 24, no. 9, 1 September 1987 (1987-09-01), pages 975-980, XP023681406, ISSN: 0161-5890, DOI: DOI:10.1016/0161-5890(87)90009-5 [retrieved on 1987-09-01] abstract; p. 977, right-hand column-end</td>
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Further documents are listed in the continuation of Box C.

See patent family annex.

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* Document member of the same patent family

Date of the actual completion of the international search: 15 February 2011

Date of mailing of the international search report: 24/02/2011

Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV RIJSWIJK Tel: (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer:
Renggli, John

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